université PARIS-SACLAY

FACULTÉ DE PHARMACIE

Hepatitis Viruses

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Master 1 D2HP

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Hepatitis: definition and etiology

- *Hepar*: Liver suffix *-itis*: inflammation
  → Liver inflammation

- non-infectious etiologies:
- Alcohol
- Drugs
- auto-immune hepatitis

- infectious etiologies:
- Bacteria: brucellosis, leptospirosis, typhoid
- Parasites: malaria
- Viruses: **hepatitis viruses**, herpesviruses (CMV, HSV, EBV), dengue virus…

Viral hepatitis burden

Source : Global Burden of Disease et estimations de l'OMS/ONUSIDA, voir http://ihmeuw.org/3pms ; http://ihmeuw.org/3pmt (consultés le 2 avril 2016).

Hepatitis viruses

5 viruses infecting human: hepatitis A, B, C, D, E

Year	Virus	Methodology	Reference
1965/1968	HBV	Serology	[3, 5]
1973	HAV	IEM (stool)	[1]
1977	HDV	Serology, IF (liver)	[16]
1983	HEV	Serology, IEM (stool)	[19]
1989	HCV	Cloning (liver)	[10]

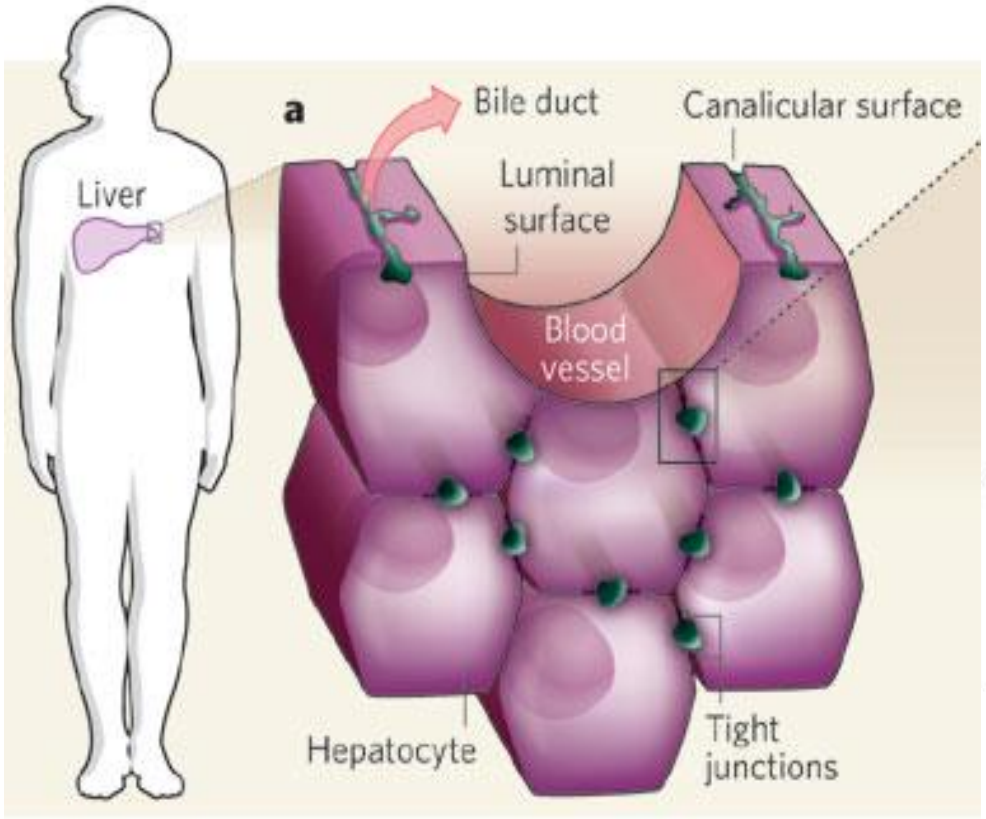
IEM = Immune electron microscopy; IF = immunofluorescence.

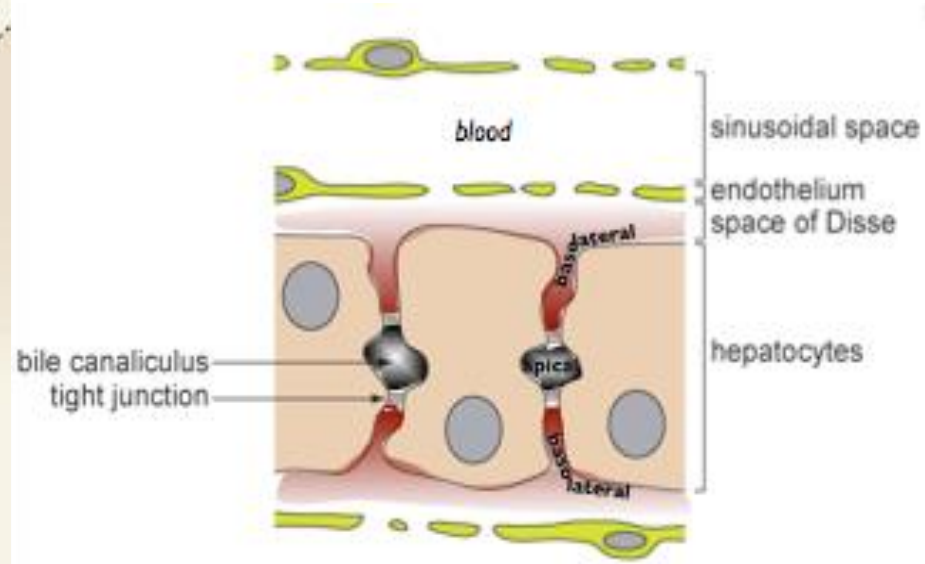
Blum, Digestive Diseases, 2016

Hepatitis viruses

Virus	Family	Genome	Transmission	Chronicity risk
Hepatitis A	*Picornaviridae*	ssRNA (+)	Fecal-oral	no
Hepatitis B	*Hepadnaviridae*	dsDNA	mother-to-child sex parenteral	Adults: ~5-10% Newborns: ~90%
Hepatitis C	*Flaviviridae*	ssRNA (+)	Bloodborne	~70-90%
Hepatitis D	*Kolmioviridae*	ssRNA (-)	Similar to HBV	yes
Hepatitis E	*Hepeviridae*	ssRNA (+)	Fecal-oral	Rare (immunocompromised patients)

Hepatitis viruses are hepatotropic





Liver functions: metabolism (including lipids), protein synthesis (coagulation factors, albumin, lipoprotein,…), storage (iron, vitamins,…), production of bile, drug metabolism...

Viral hepatitis: clinical presentations

- asymptomatic (acute infections +++)

- acute hepatitis

- fever, malaise, fatigue, headache, loss of appetite, vomiting, diarrhea, and abdominal pain.
- Icterus/jaundice

- fulminant hepatic failure : rapid development of jaundice and hepatic encephalopathy in a person without a history of liver disease

Viral hepatitis: clinical presentations

Acute hepatitis A and E

- **HAV :** cause only acute hepatitis
- Usually asymptomatic (children in high endemicity country+++). Around 10 000 death/year
- Vaccine available

- **HEV :** cause mainly acute hepatitis
- Per year: 20 million HEV infections worldwide, 3.3 million symptomatic cases of hepatitis E (44 000 deaths in 2015)
- Hepatitis E is more severe in pregnant women and immunocompromised patients
- most common in East and South Asia
- Vaccine available in China

Viral hepatitis: clinical presentations

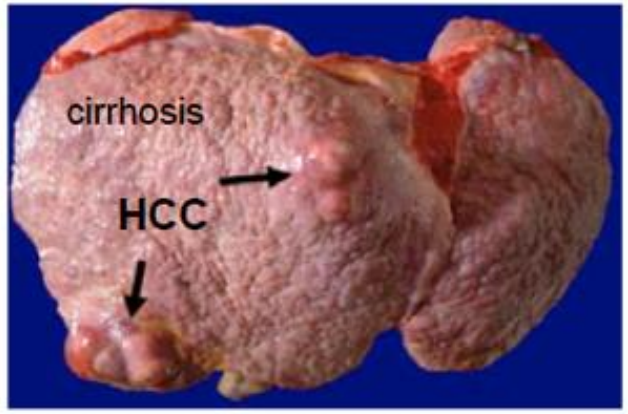
- Chronic hepatitis:

the virus persist > 6 months in the organism and liver injury is caused by immune reaction/chronic inflammation

healthy liver

cirrhosis

functional hepatocytes replaced by connective tissue



primary liver carcinoma
(hepatocellular carcinoma, HCC)

Immunopathogenesis, viruses are not cytotoxic → years → years

15 - 25% of chronic carriers within 20 - 40 years;

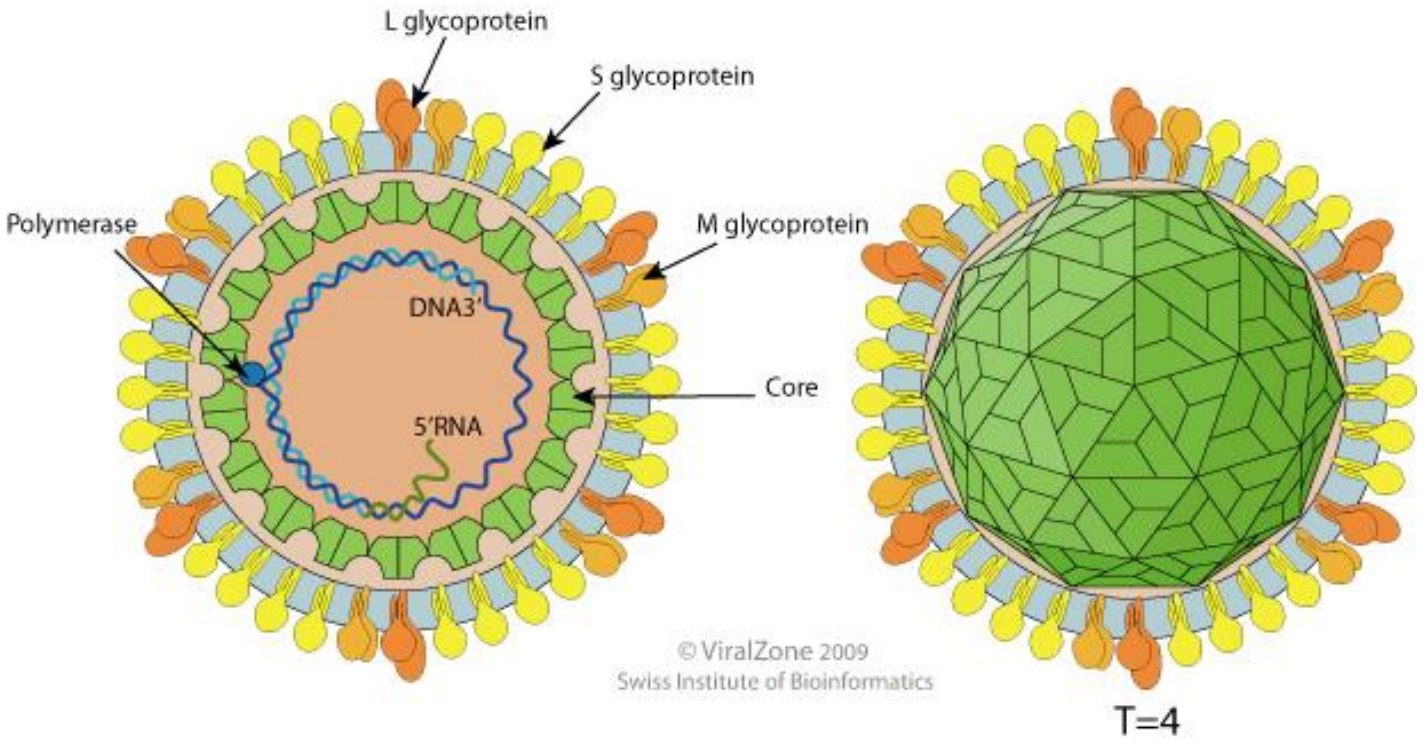
How do you assess liver disease?

- Physical examination and biochemical parameters
- Biochemical parameters: including alanine aminotransferase (**ALT**)
- Fibrosis markers: non-invasive markers of fibrosis (elastography or biomarkers) or liver biopsy in selected cases

HEPATITIS B

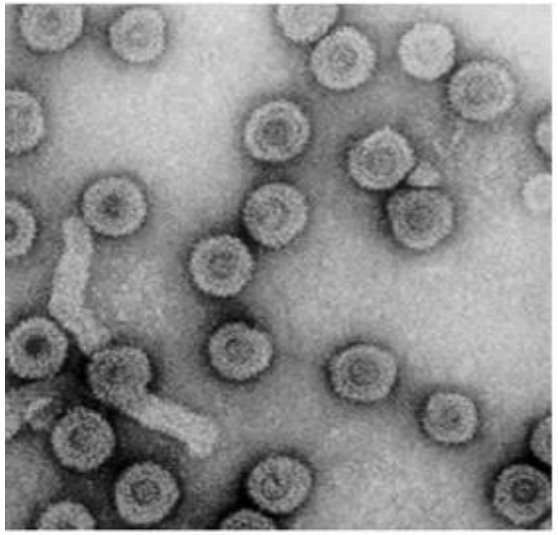
Hepatitis B virus (HBV)

- Family: *Hepadnaviridae*
- Genus: *Orthohepadnavirus*

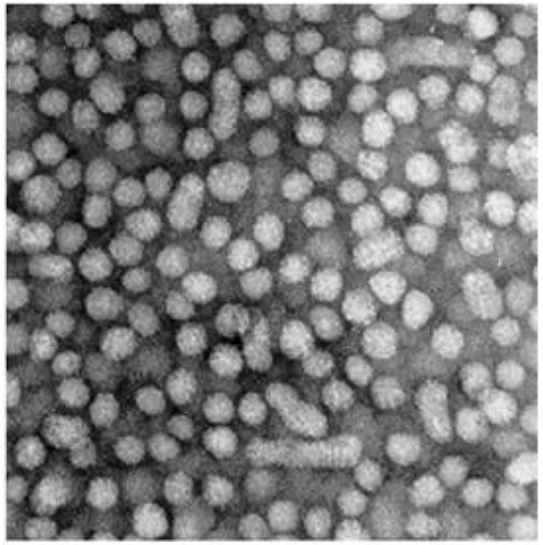


- Structure: enveloped, icosahedric capsid
- Partially dsDNA circular genome, about 3.2 kb
- One of the smallest virus infecting human (42nm)

Hepatitis B: viral particles



Dane particles (virions)
10^9



spheres
10^{13}

filaments
10^{10}

infectious particles

non-infectious particles (empty envelopes)

History of HBV research

Identification of NTCP as receptor for HBV 2012

2008 **Licensing** of Tenofovir

First reverse-transcriptase inhibitors 1998

Approval of Interferon therapy 1992

1986 **Hepatitis B virus recombinant vaccine**

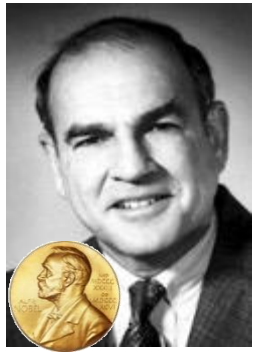
1968 **Identification of HBsAg as part of virus causing serum hepatitis**

Description of the Australia antigen (HBsAg) 1963

Description of post-transfusion hepatitis

1943

Beeson



Bloomberg

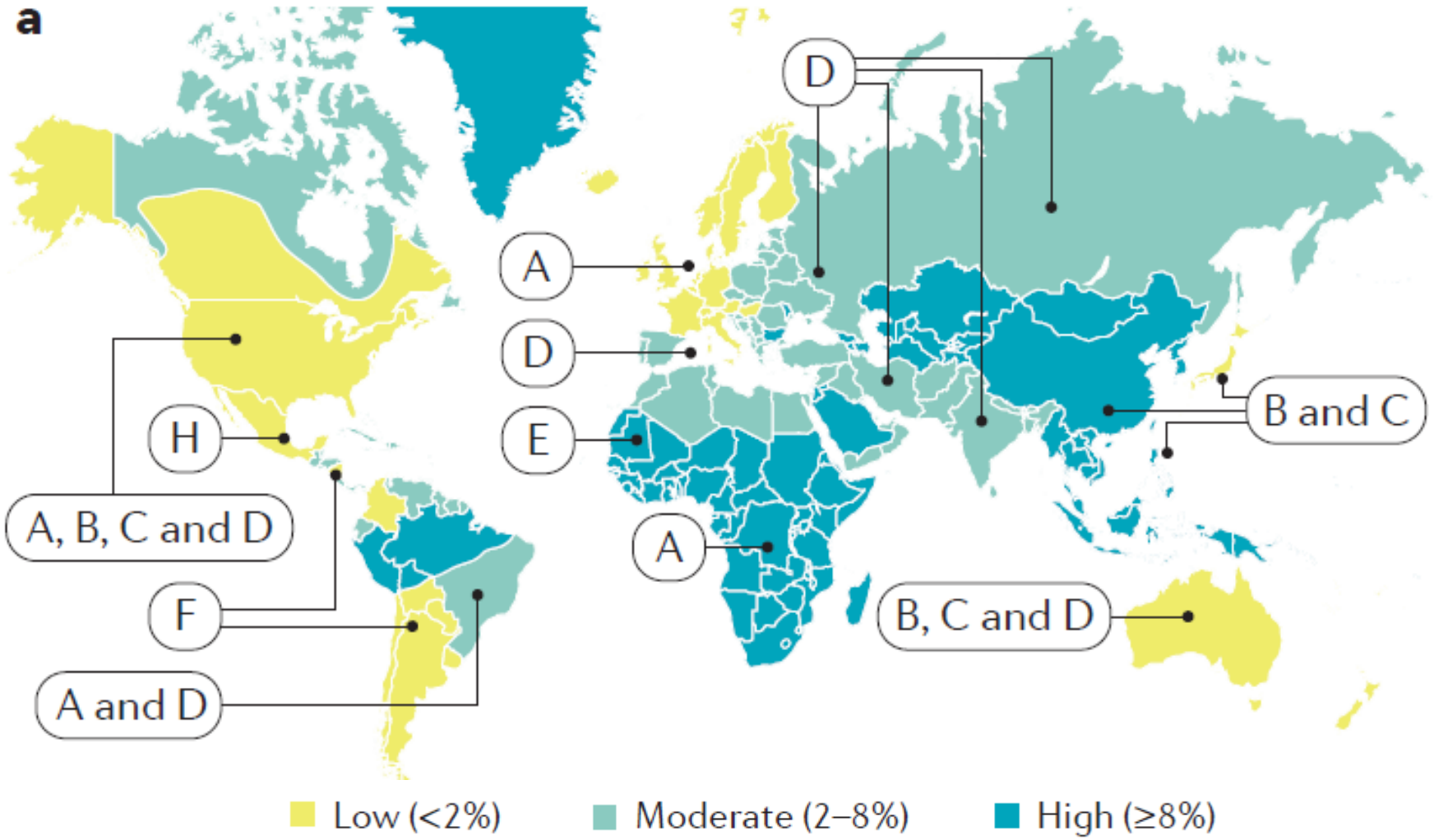
Prince

Valenzuela

Chisari

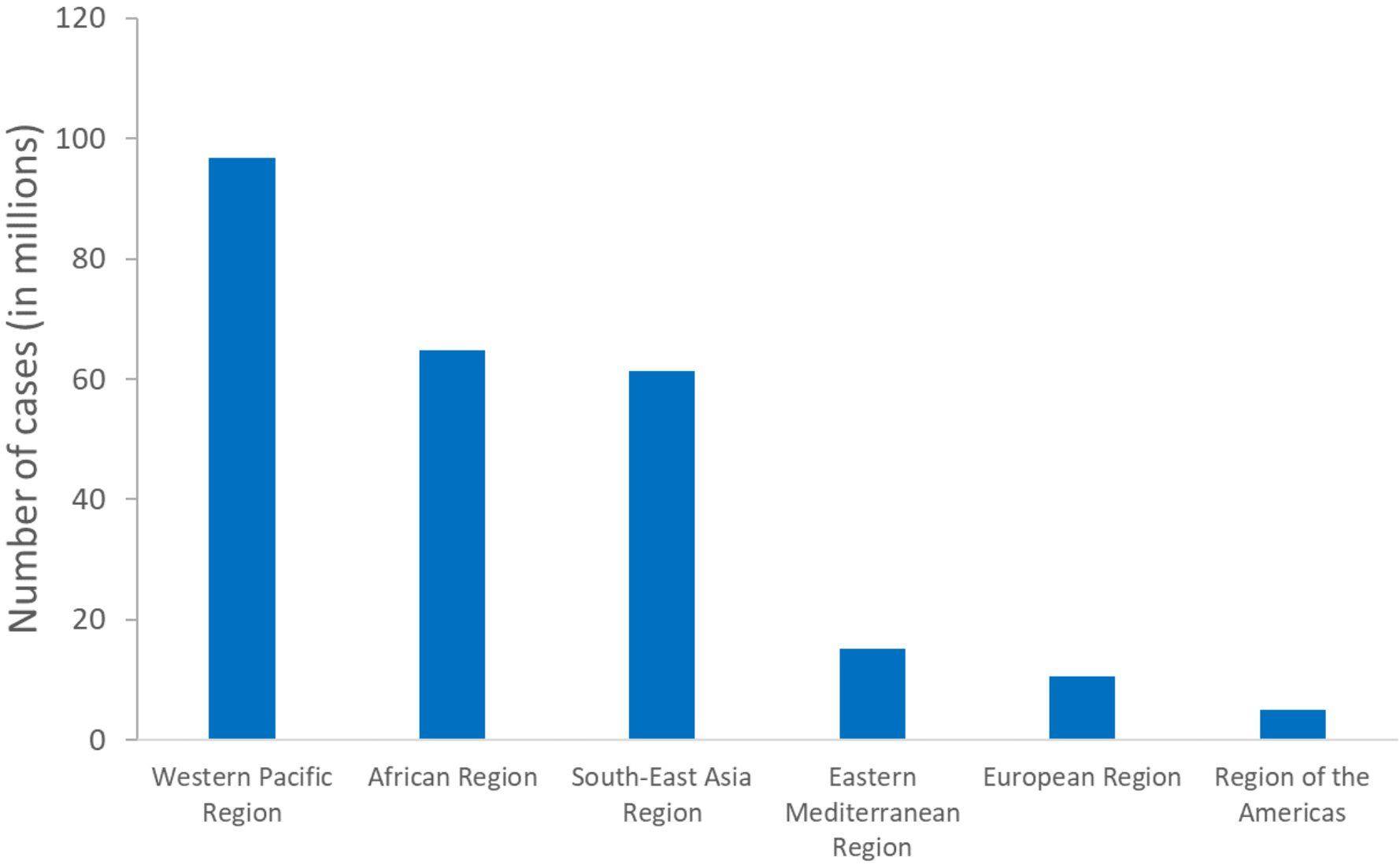
Hepatitis B: epidemiology

10 genotypes: A to J



Yuen et al. *Nat Rev Disease Primers* (2018)

Hepatitis B: epidemiology



GLOBAL HEPATITIS REPORT, 2024, WHO

Hepatitis B: epidemiology

According to WHO:

- 2022: **254 million people** chronically infected by HBV
- **1.1 million deaths in 2022** (cirrhosis and hepatocellular carcinoma)
- HBV accounts for around 45% of cases of HCC and 30% of cirrhosis
- **1.2 million of new infections**
- **1/3 of the population** has already been infected
- 13% of people living with HBV are aware of their infection
- 3% of people living with HBV are treated

Hepatitis B: epidemiology

- In France (2004):
- **300 000** people with chronic hepatitis B (prevalence = 0.68 %)
- anti-HBc antibodies = **8%**

- “Barotest” study (2016) :
- HBs antigen prevalence = 0.30% (**135 000** people infected)
- **only 17,5%** of infected people were already diagnosed!!

Hepatitis B: transmission

- infect only humans : viral reservoir
- Contamination by contact with infected blood, seminal and vaginal fluids
- The virus can survive up to 7 days outside the body
- Incubation : 75 days on average (30-180)

Hepatitis B: transmission

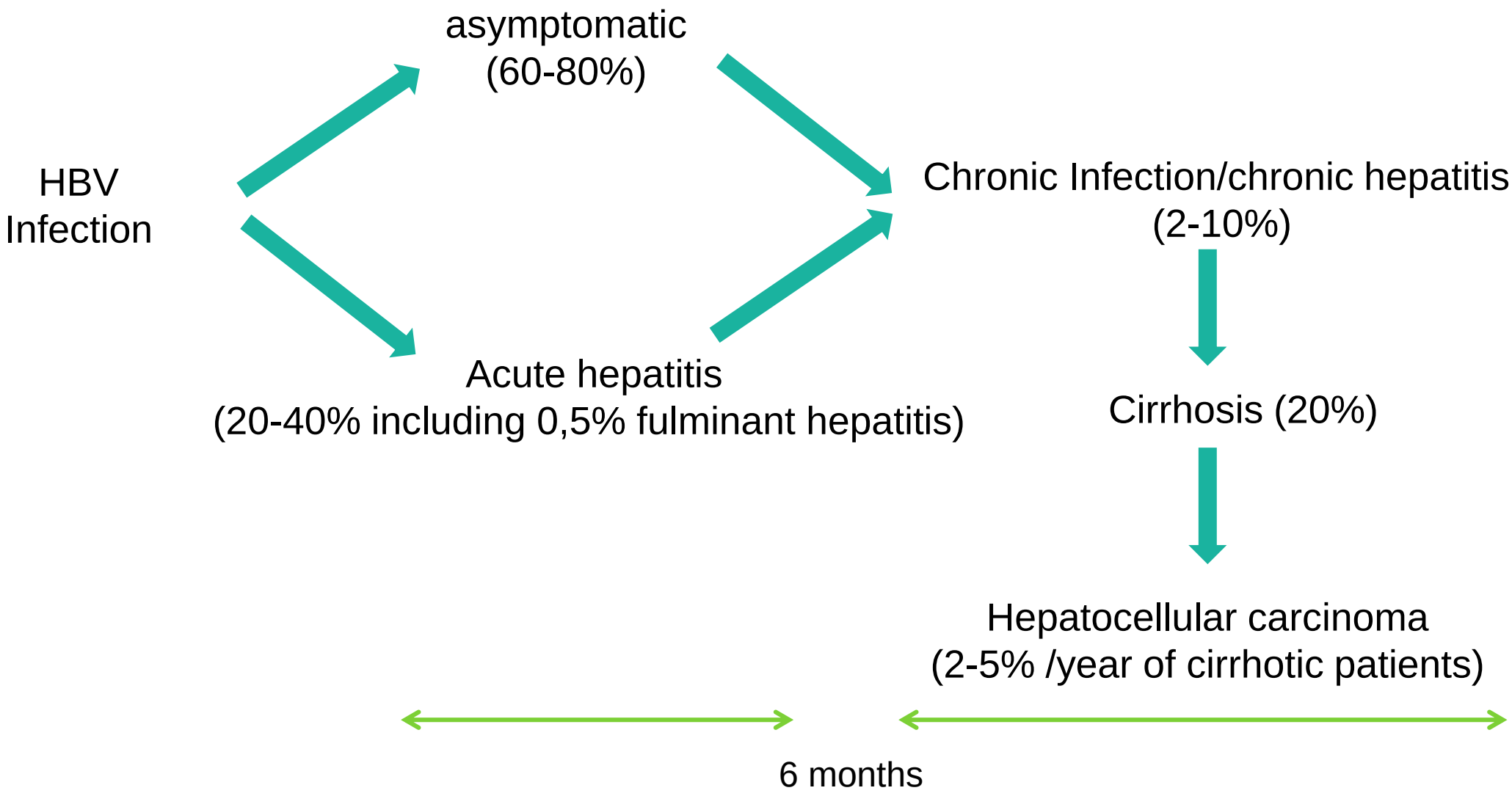
- Vertical transmission (perinatal)
  - From infected mother to child, at birth mostly

- Parenteral transmission (contact with infected blood)
  - Injected drugs
  - Tattooing, piercing,…
  - Transfusion, reuse of needles and syringes

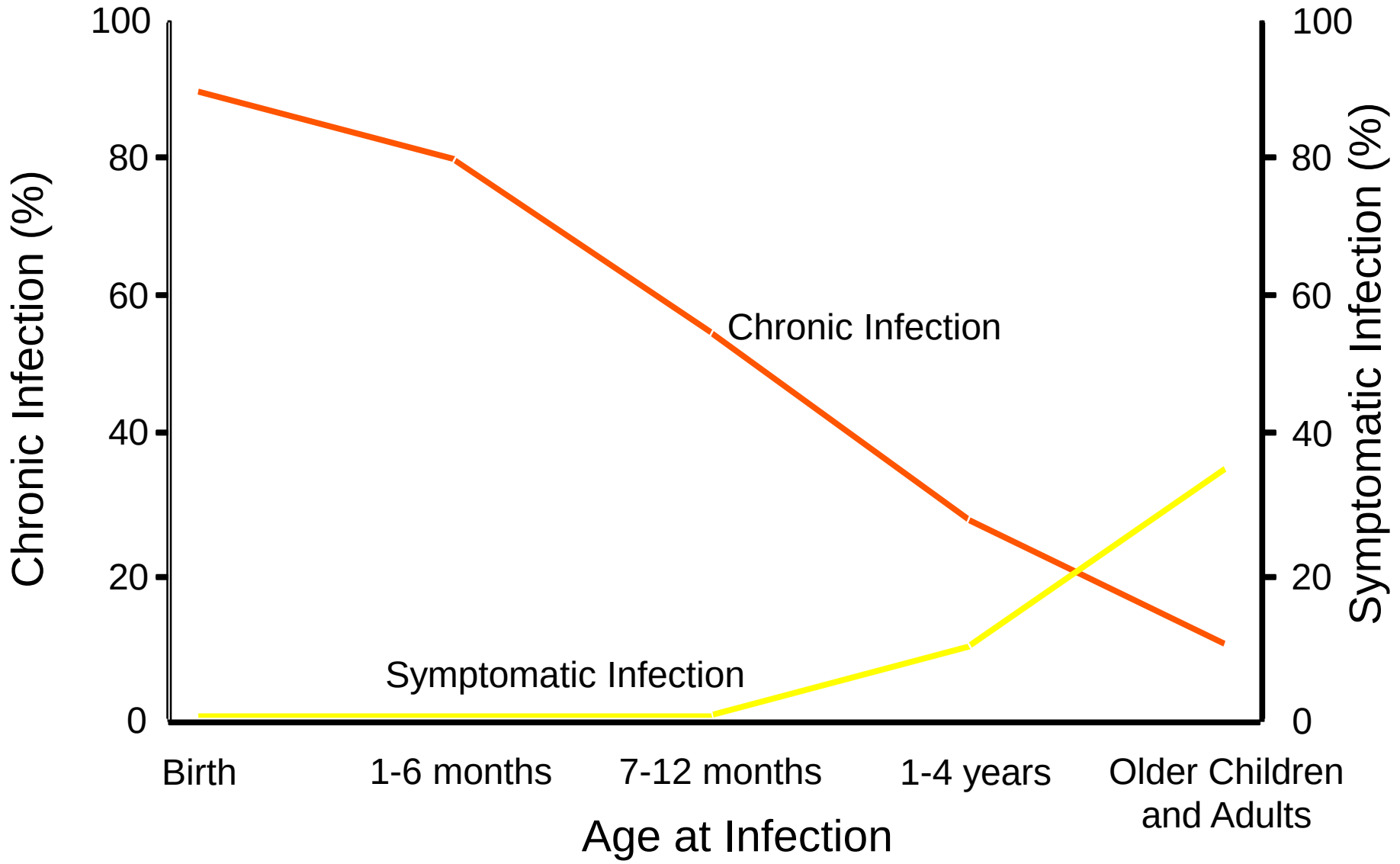
- Sexual transmission

- Other horizontal transmission
  - including household, intrafamilial and especially child to-child

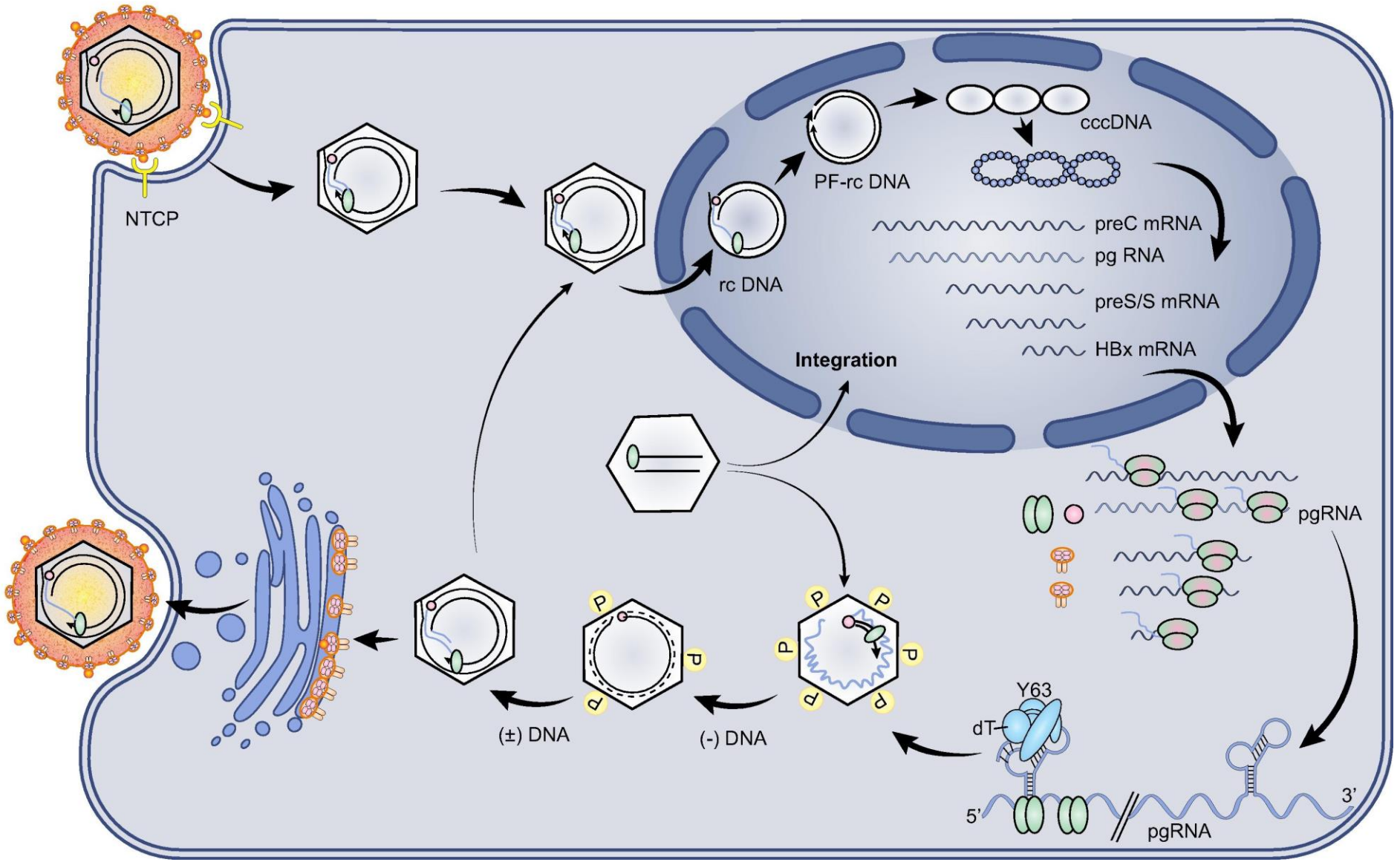
Hepatitis B: natural history of infection



Hepatitis B: natural history of infection



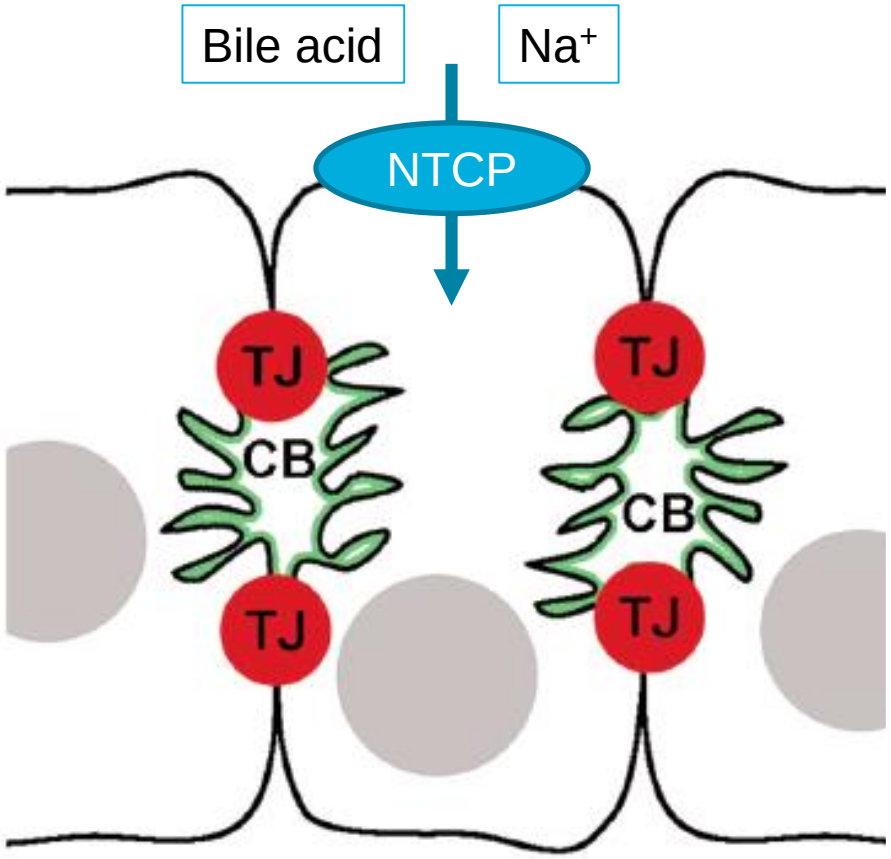
HBV: replication cycle



Tong et al, Journal of Hepatology 2016

HBV receptor: NTCP

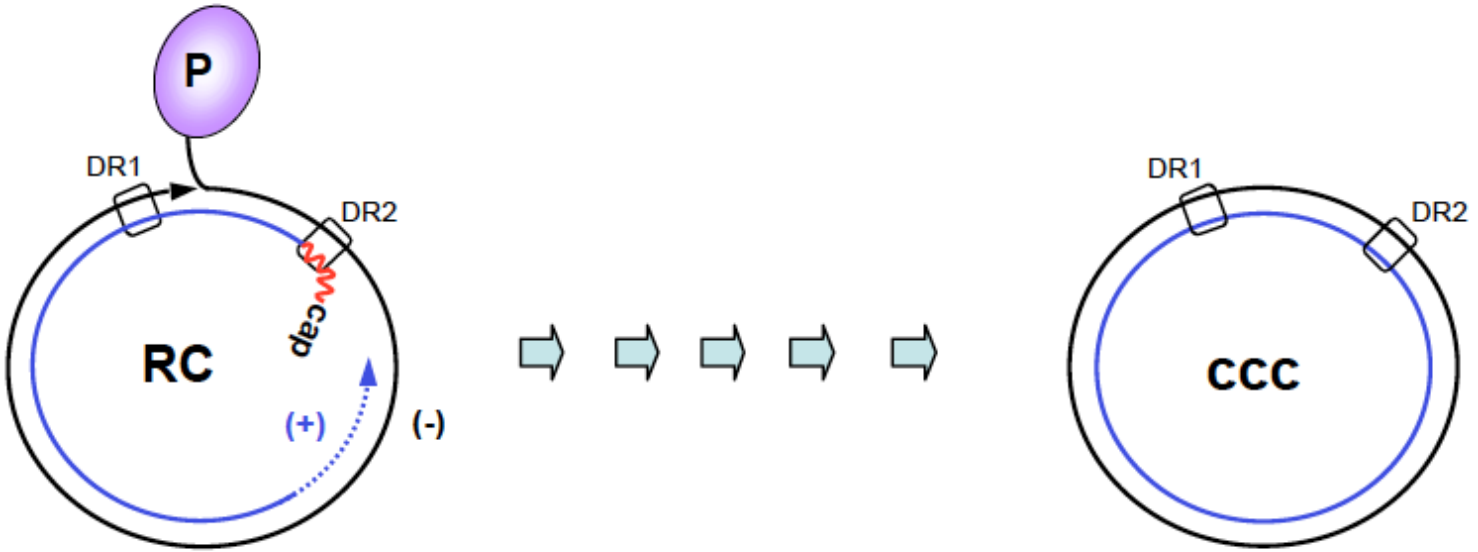
NTCP : Sodium-taurocholate cotransporting polypeptide transmembranous protein
→ hepatic transporter
→ expressed mostly at the basolateral side of hepatocyte



→ Coherent with the hepatic tropism of HBV and blood transmission

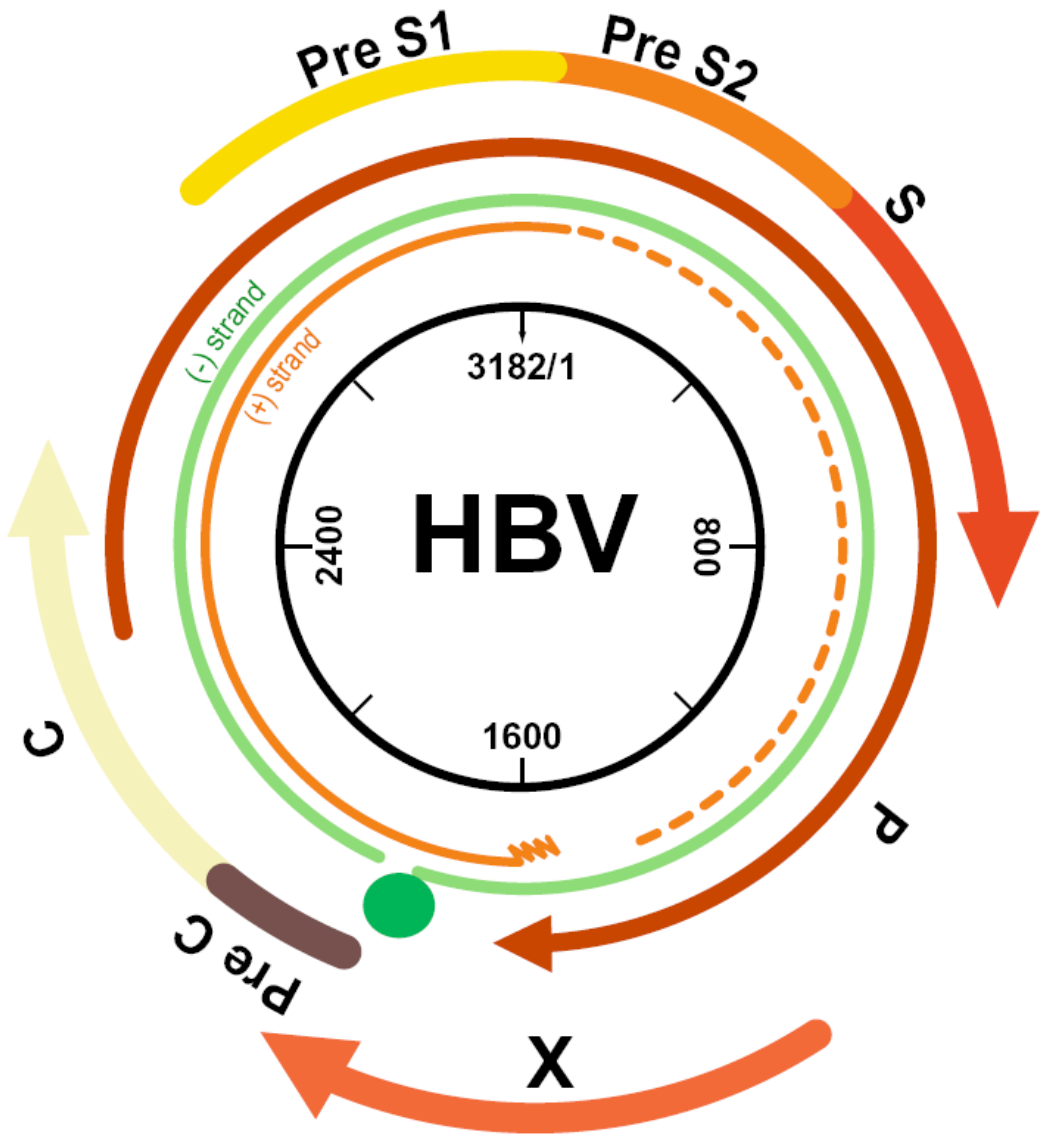
HBV: cccDNA

In the nucleus, viral genome is converted in a “minichromosome” called **cccDNA** for **covalently-closed circular DNA**
cccDNA is the matrice for viral RNA transcription



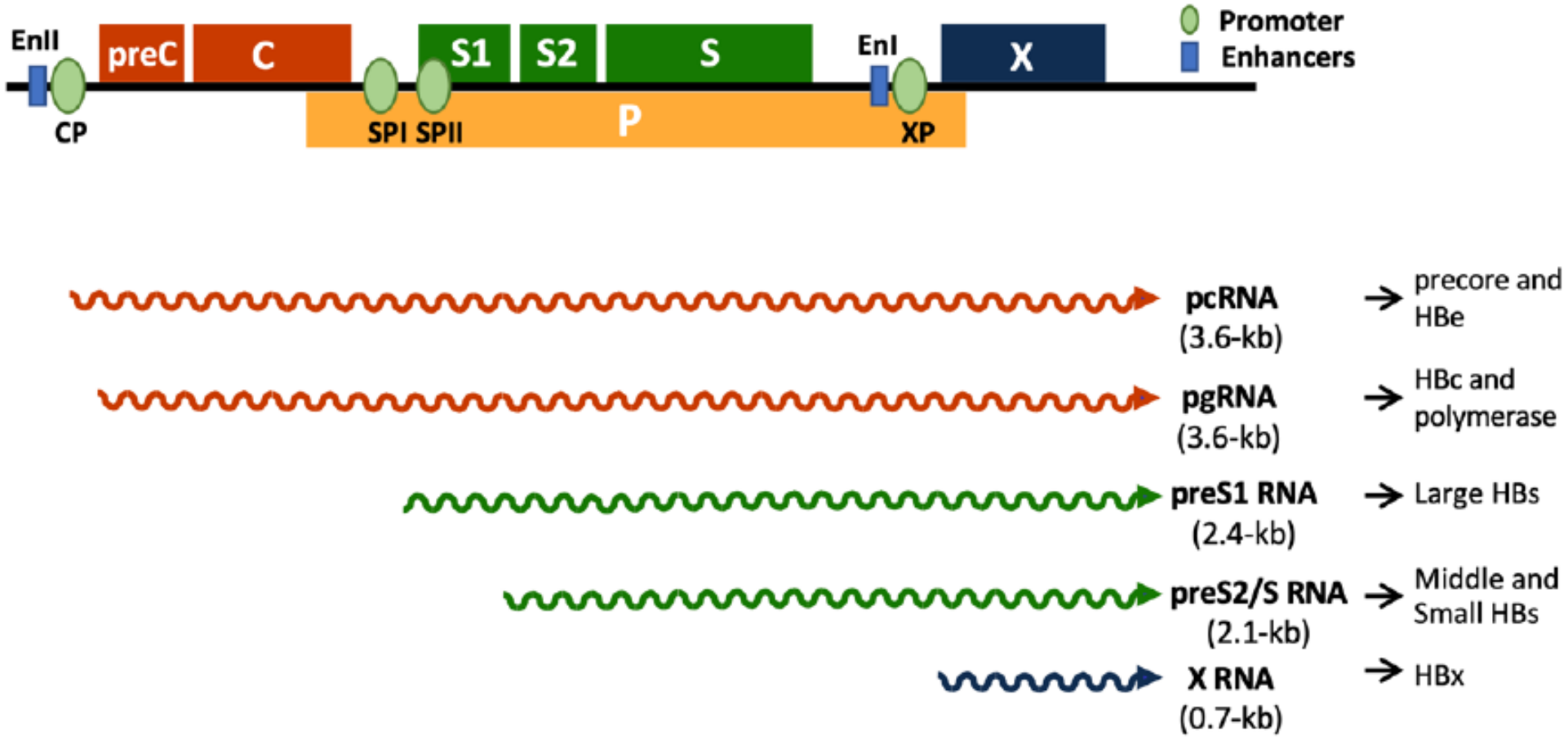
cccDNA persists in the nucleus of hepatocyte
→ cannot be cleared from the organism
→ can reactivate (immunocompromised patients)

HBV: genome organization



- Smallest DNA genome
- Each nucleotide has coding function
- Overlapping ORFs

HBV ORFs and RNAs

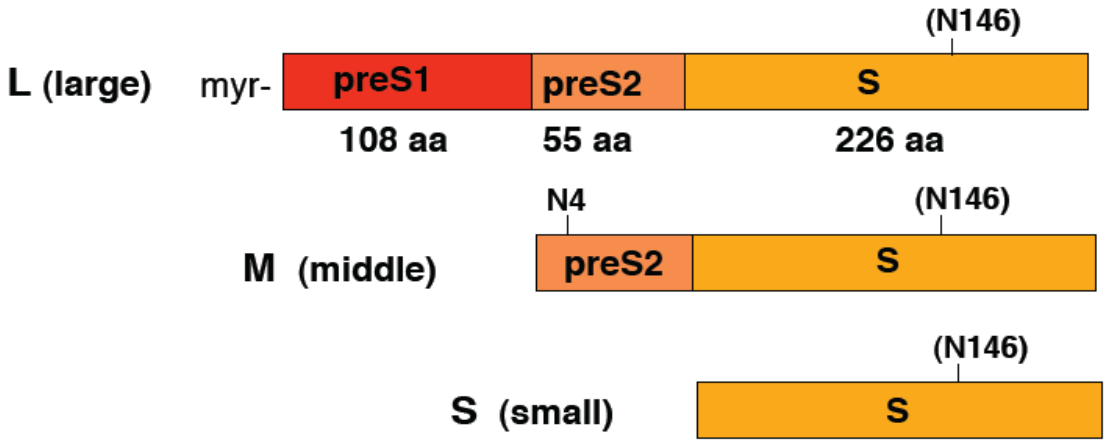


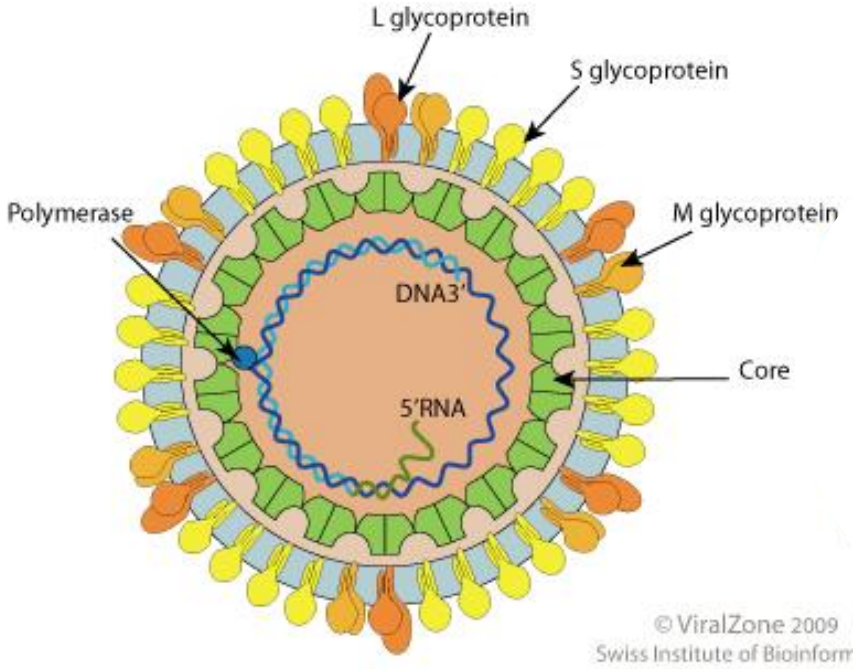
Li et al, Viruses 2018

HBV proteins

Gene S (preS1, preS2 and S)
= envelop protein: L, M and S

➡ HBs antigen (HBsAg)

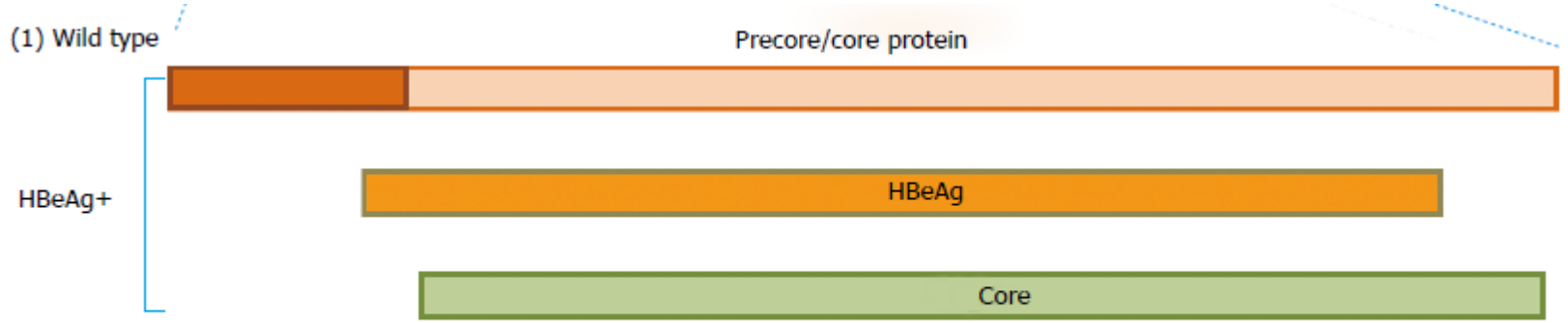




HBV proteins

Gene C (preC and C)

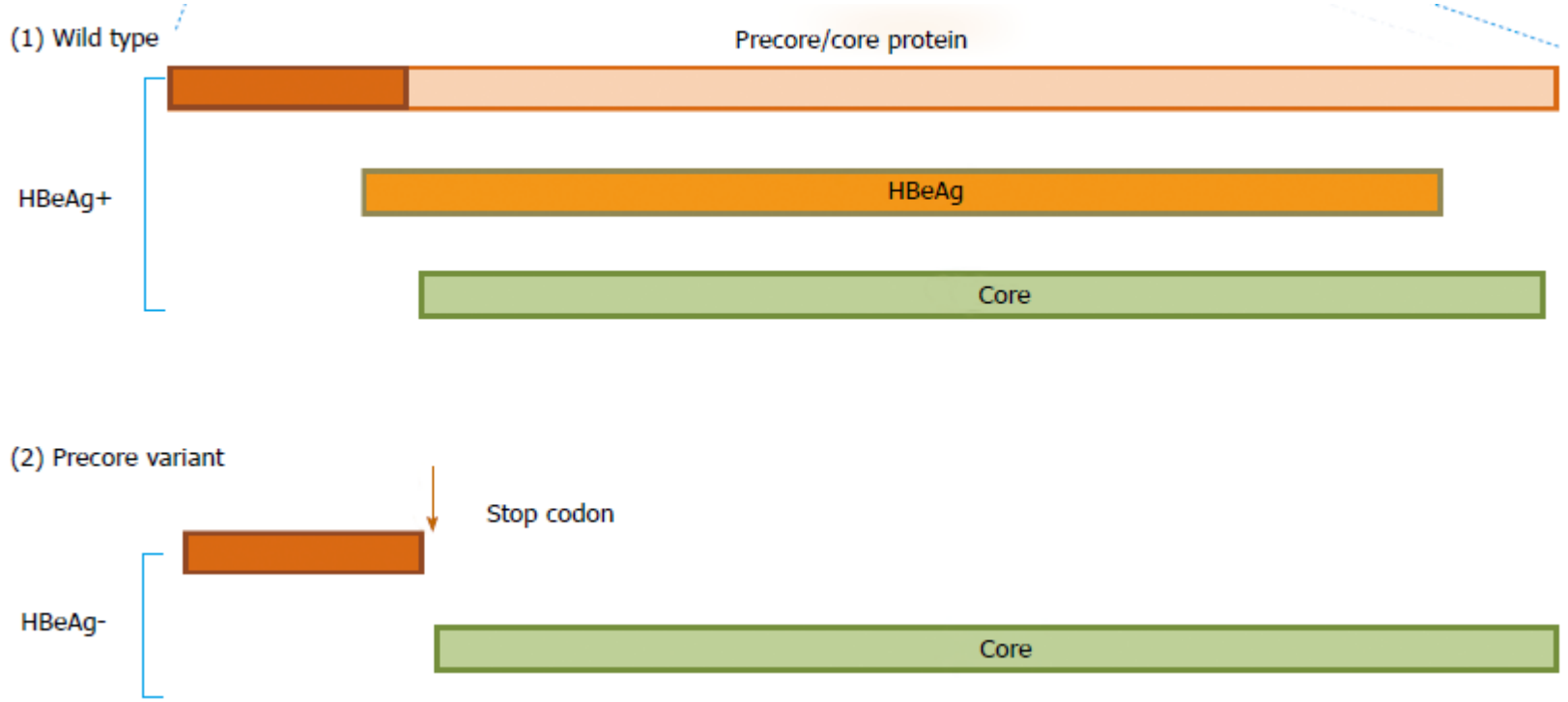
-HBc antigen: capsid protein
-HBe antigen: secreted protein, function?



Alexopoulou et al, World J Gastroenterol 2014

HBV proteins

precore mutant :
introduction of the stop codon mutation leads to the abrogation of HBeAg production

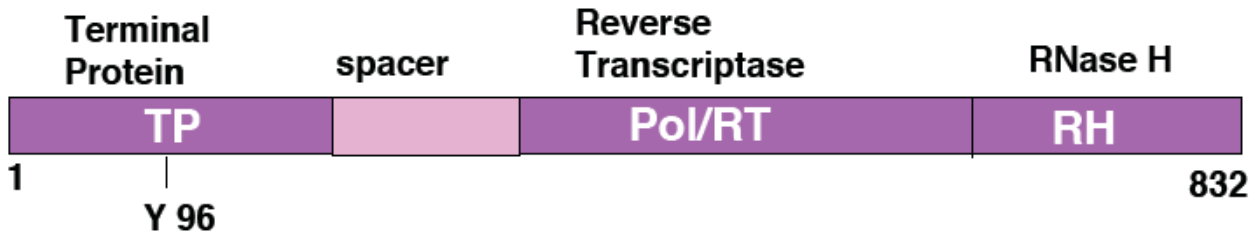


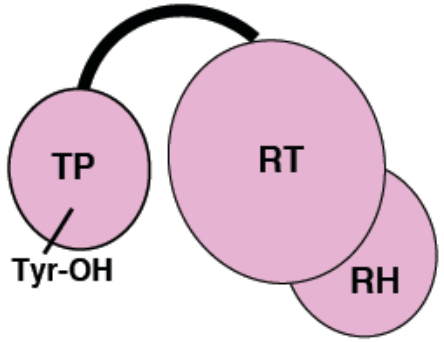
Alexopoulou et al, World J Gastroenterol 2014

HBV proteins

Gene P : polymerase

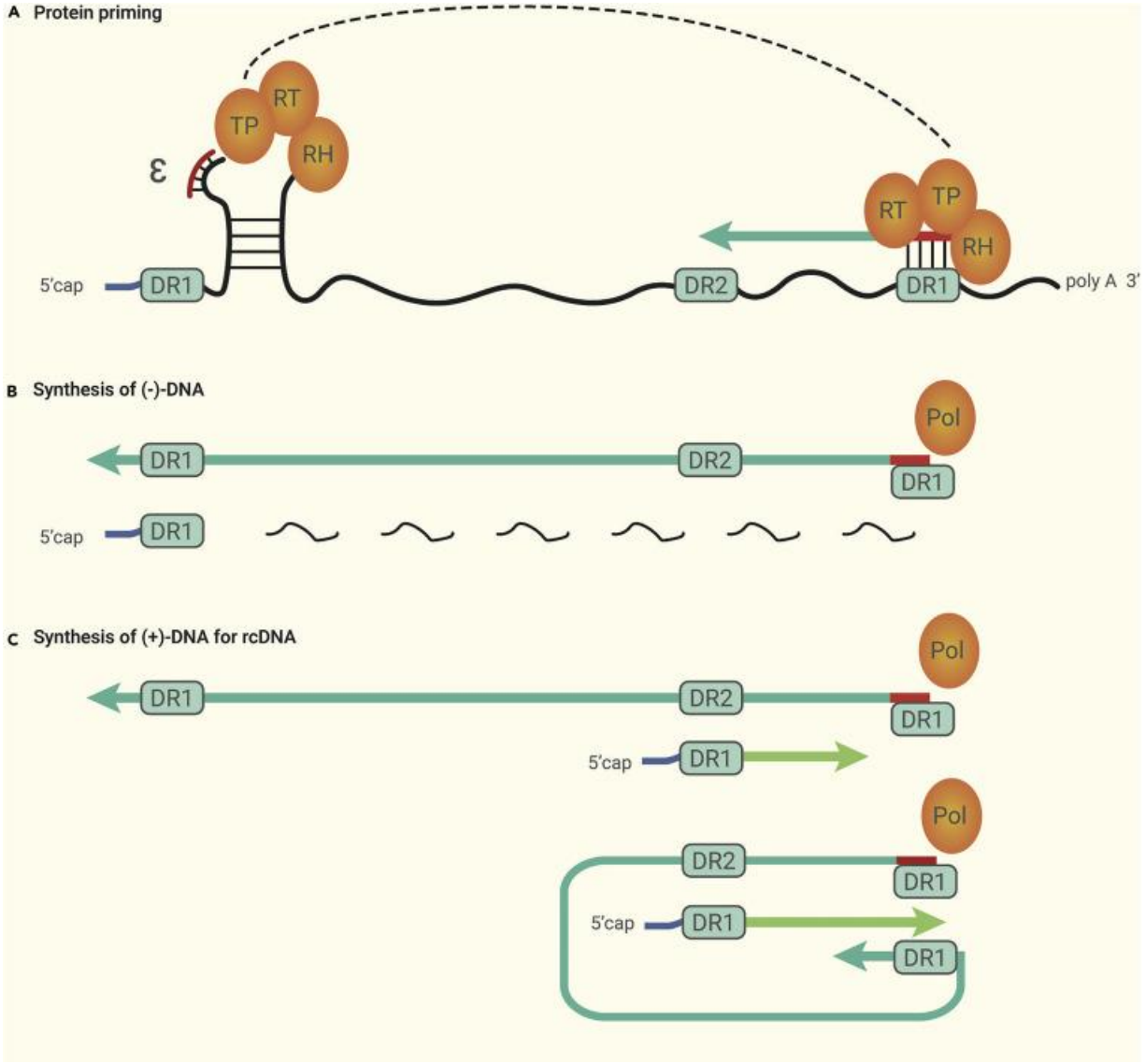
RNA-dependant DNA polymerase (= reverse transcriptase) and RNase H





Target of therapeutic : reverse transcriptase inhibitors initially developed for HIV

HBV reverse transcription



- synthesis of the first DNA strand (-) from pre-genomic (pg) RNA
- pgRNA is degraded by the RNase H domain
- a undegraded RNA oligo serves as the positive strand DNA primer
- DNA (+) is incomplete

HBV proteins

Gene X : HBx

- Trans-activator activity on viral and cellular genomes (involved in carcinogenesis)
- Regulates host defenses, viral replication,…

Hepatitis B: viral markers

- **HBs antigen / anti-HBs antibodies**

– First Ag described, can be detected in blood and the cytoplasm of hepatocytes

– **HBsAg persistance > 6 month = chronic hepatitis**

– anti-HBs antibodies : protection (vaccine = recombinant HBsAg)

- HBc antigen **/ anti-HBc antibodies**

– Ag not detected in blood, but found in hepatocyte

– anti-HBc antibodies in serum: are not protective

– Anti-HBc IgM are used to diagnose acute infection

Hepatitis B: viral markers

- **HBe antigen**

Detected in the blood → **replication marker**

- **anti-HBe antibodies**

- Detected in persons with no or lower levels of HBV replication
- Ag HBe disapear when anti-HBe are produced (seroconversion)
- ! precore mutants

- **HBV DNA (in serum)**

- HBV DNA correlates with levels of circulating viral particles = **measure viral replication**

Hepatitis B: diagnostic techniques

- **Direct diagnosis**

  - detection of antigens in serum : HBsAg and HBeAg by laboratory-based immunoassay (ELISA) (also RDT for HBsAg)

  - HBV DNA in serum : PCR (quantification = viral load)

- **Indirected diagnosis :** ELISA

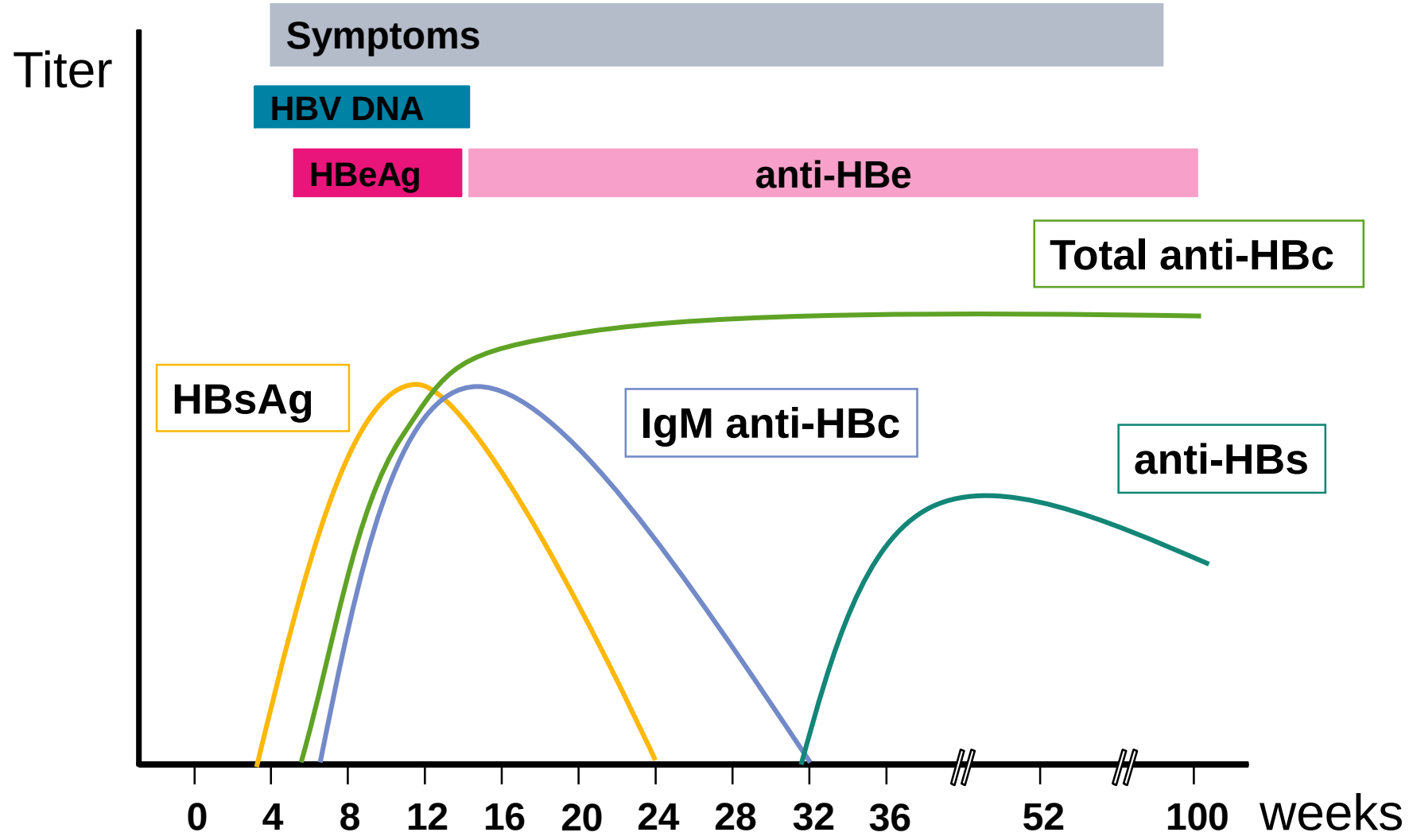
  - anti-HBs antibodies : vaccination or resolved infection

  - anti-HBc antibodies:

    . IgM : acute infection

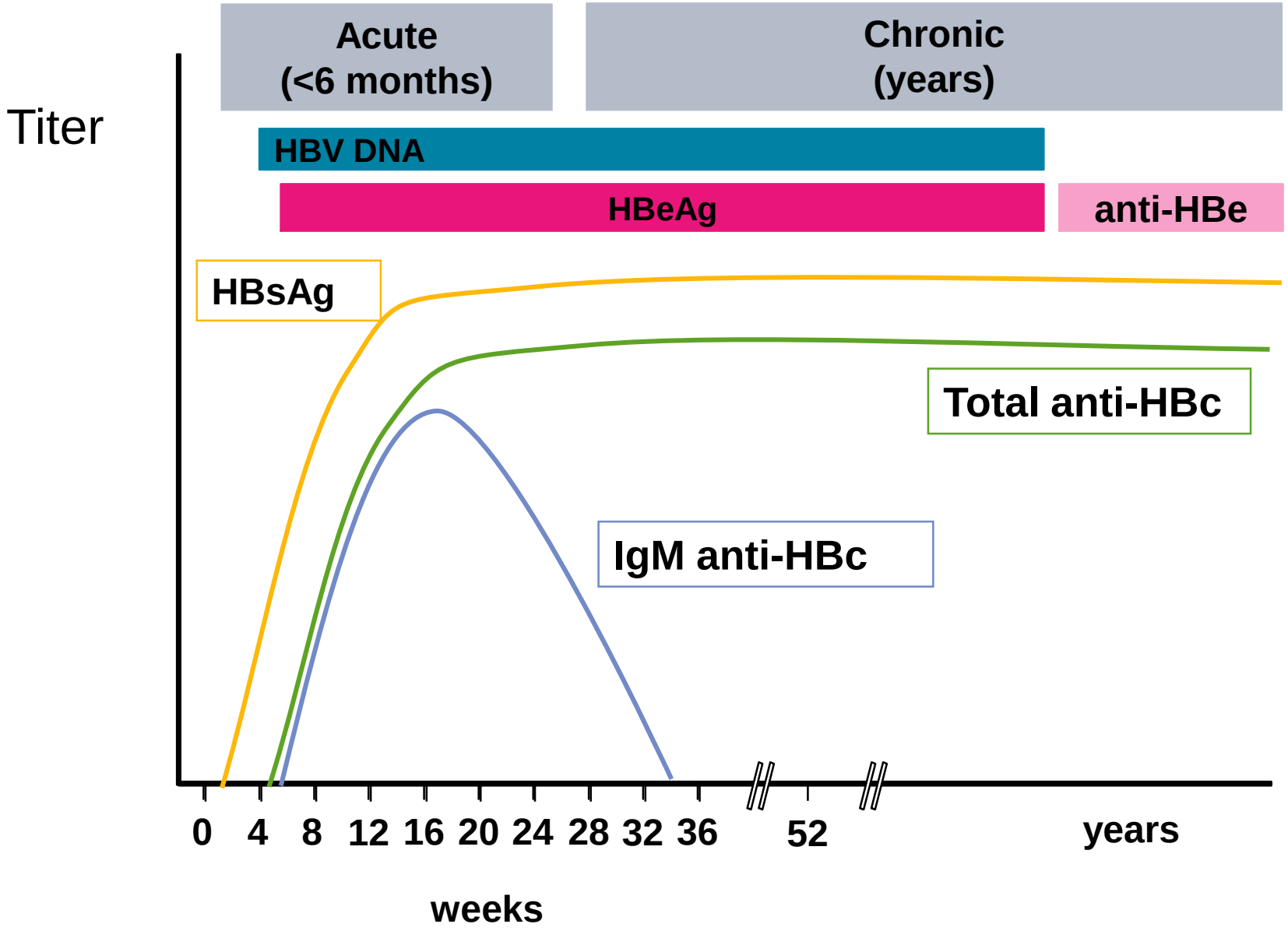
  - anti-HBe antibodies: usually a sign of positive evolution (or pre-C mutation→ measure or viral load)

Acute hepatitis B: serological course with recovery



Source : Center for Disease Control and Prevention

Acute hepatitis B: serological course with progression to chronic infection



Who and how to test

- General population testing (intermediate and high seroprevalence)
- Pregnant women (intermediate and high seroprevalence)
- Focused testing :
  - Populations most affected by HBV infection (part of a population with high HBV seroprevalence or history of exposure and/or high-risk behaviours for HBV infection)
  - Clinical suspicion of chronic viral hepatitis
  - Sexual partners, children and other family members, and close household contacts of those with HBV infection
  - Health-care workers

- Blood donors (mandatory)

- WHO guidelines : **detection of HBsAg** (Single RDT or laboratory-based immunoassay)
- In France : detection of HBsAg , anti-HBs and anti-HBc

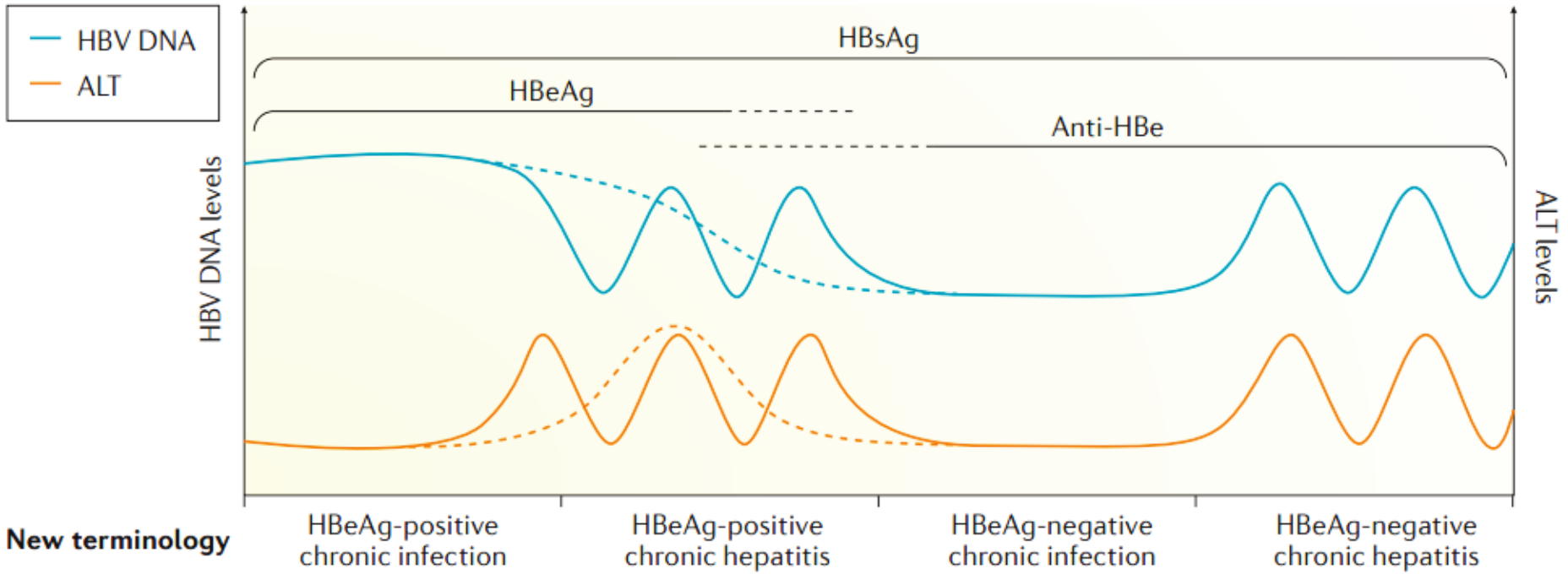
Hepatitis B testing

	HBsAg	Anti-HBs	Anti-HBc
Acute hepatitis B	+	-	+ (IgM)
Chronic infection / chronic hepatitis B	+ (> 6 months)	-	+
Resolved hepatitis B	-	+	+
vaccinated	-	+	-

if testing is positive (**HBsAg +**) :

- marker of viral replication (**HBeAg and viral DNA**)
- assessment of stage of liver disease (ALT, non-invasive tests)
- co-infections (HCV, HDV, HIV)
- other co-morbidity

HBV chronic stages

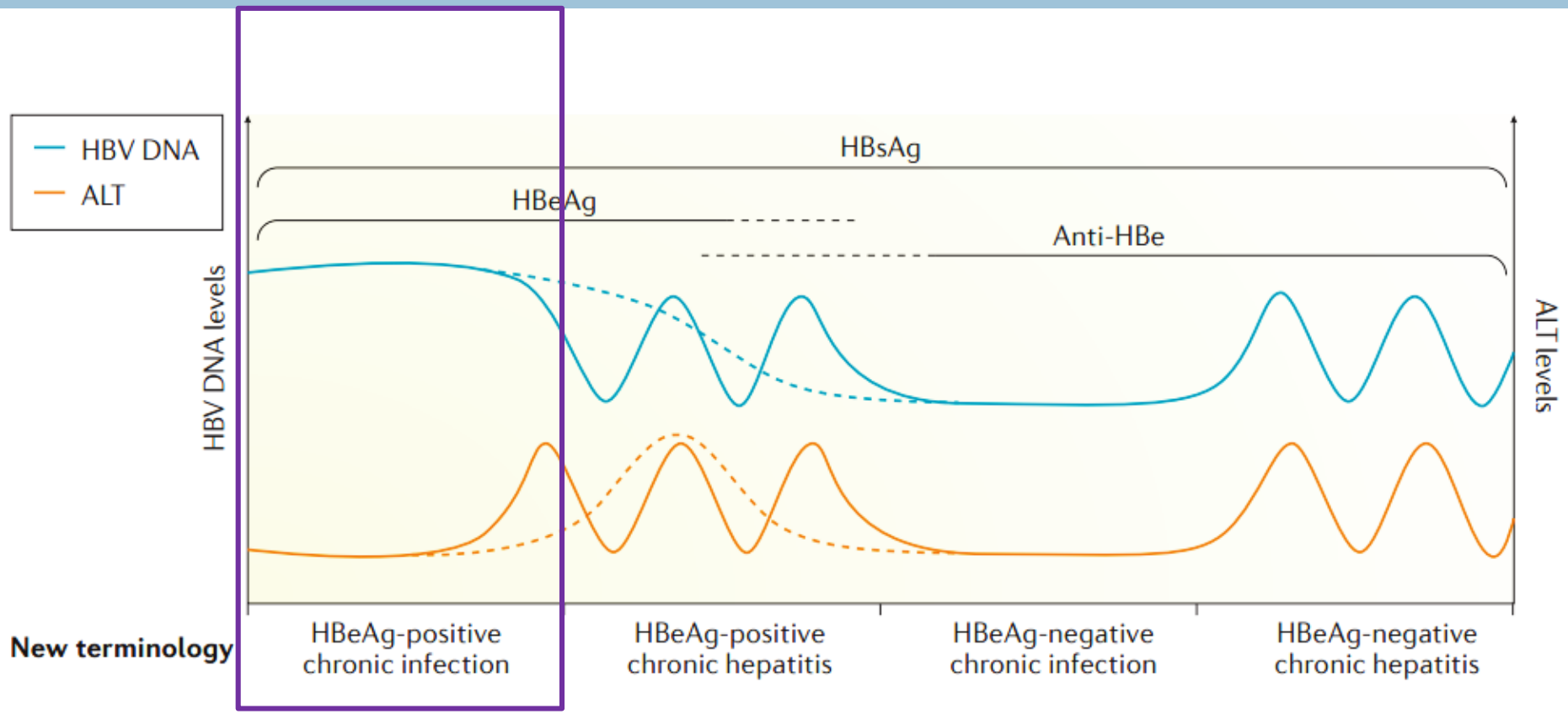


HBV markers
- HBsAg
- HBeAg / anti-HBe
- HBV DNA levels

liver disease
- ALT levels
- fibrosis markers

Yuen et al. *Nat Rev Disease Primers* (2018)

HBV chronic stages

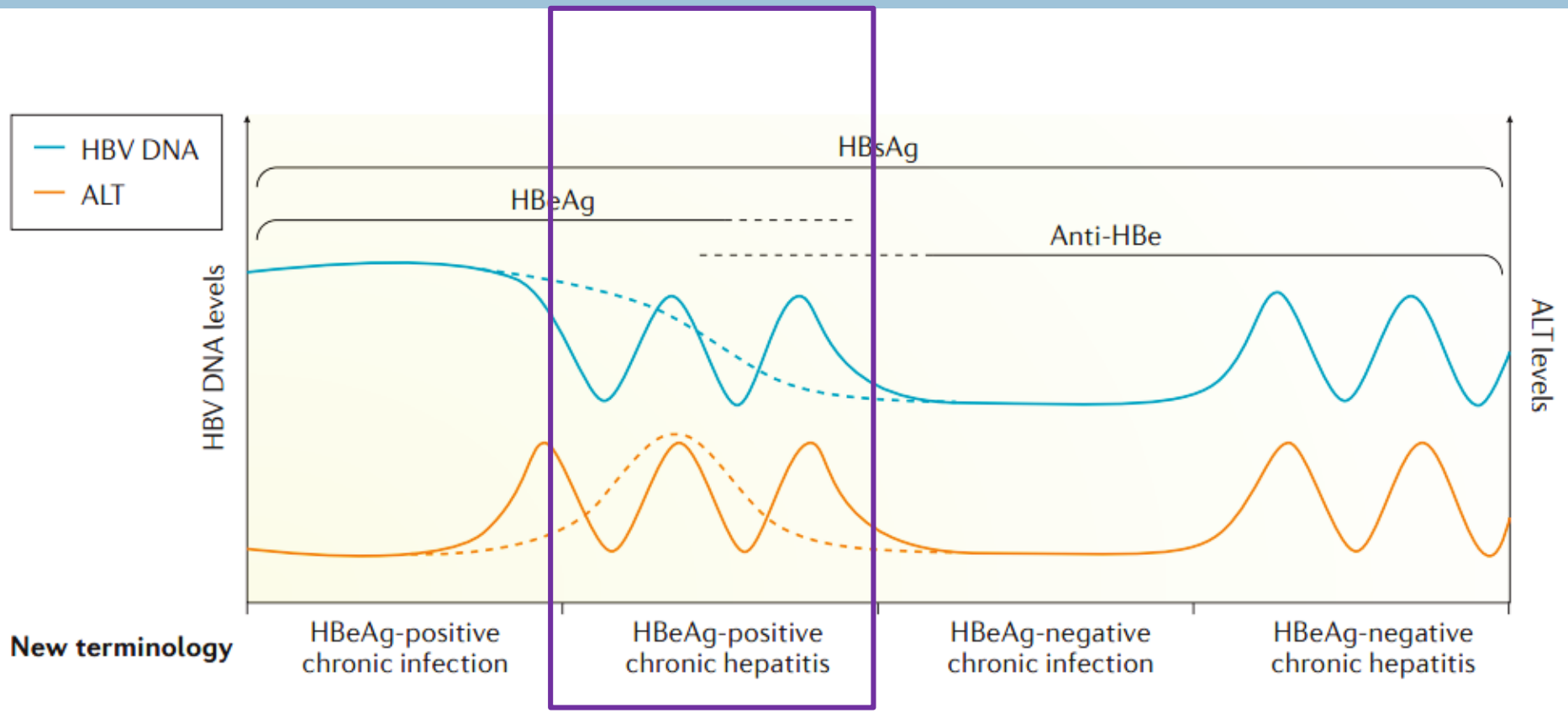


previously termed « immune tolerant» :

- active replication: HBeAg + and high HBV DNA levels >10^7 IU/ml **(= contagious ++)**
- Normal ALT levels, no fibrosis

Yuen et al. *Nat Rev Disease Primers* (2018)

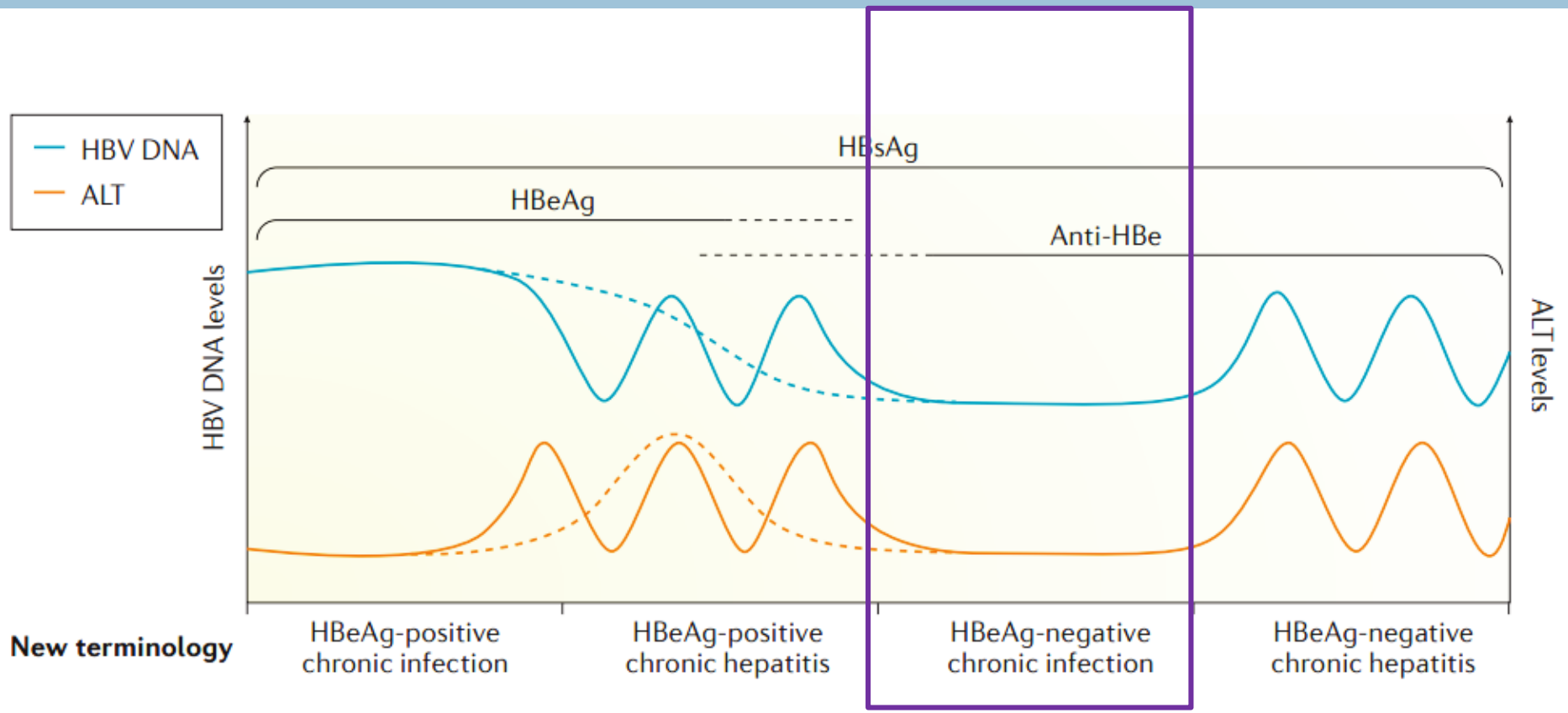
HBV chronic stages



- Active replication : HBeAg + and high DNA levels (10^4-10^7 IU/ml)
- ALT ↗, accelerated progression of fibrosis

Yuen et al. *Nat Rev Disease Primers* (2018)

HBV chronic stages

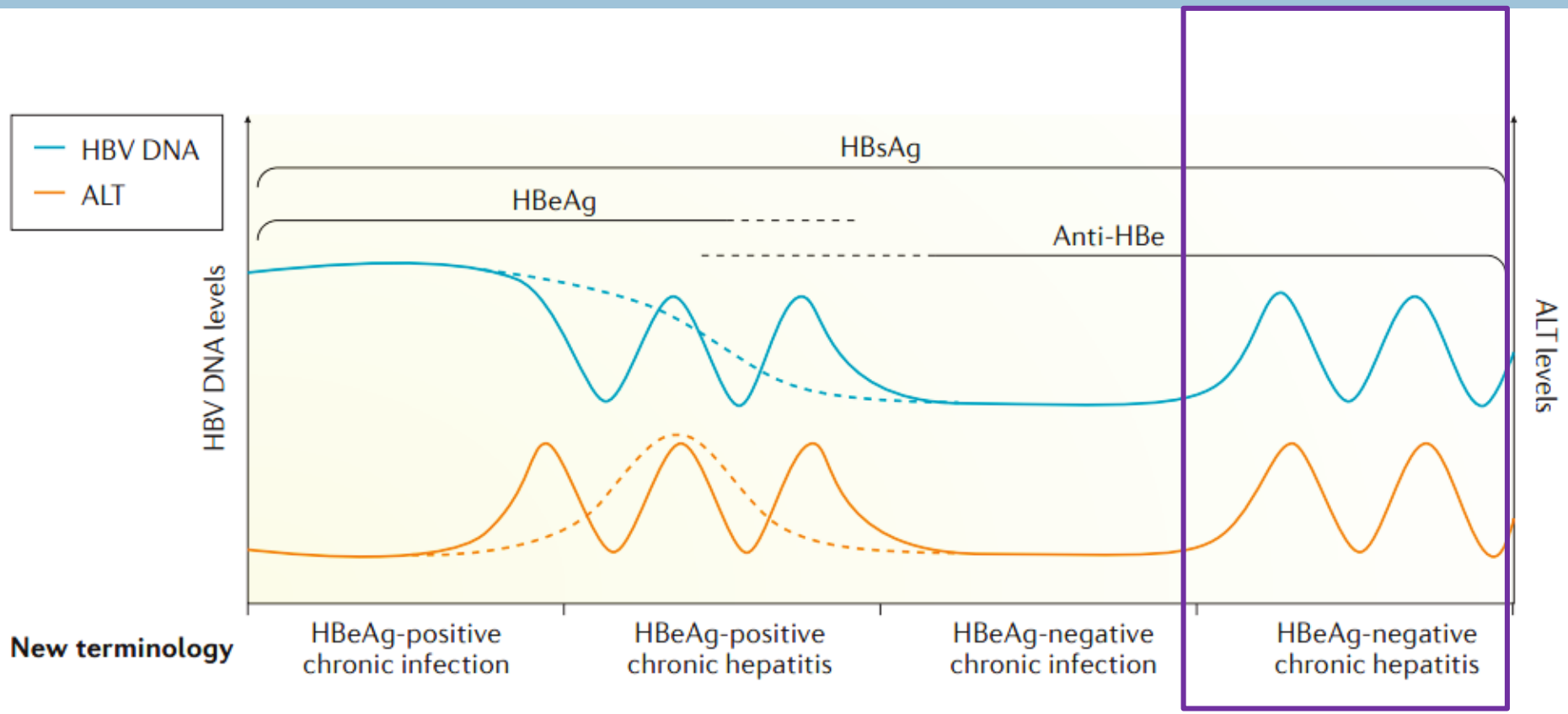


previously termed « inactive carrier » :

- no or minimal replication: HBeAg - and viral DNA undetectable or low (<2,000 IU/ml)
- normal ALT, no/low fibrosis
- in that phase, spontaneous HBsAg loss and seroconversion occurs in 1 to 3% of cases /year

Yuen et al. *Nat Rev Disease Primers* (2018)

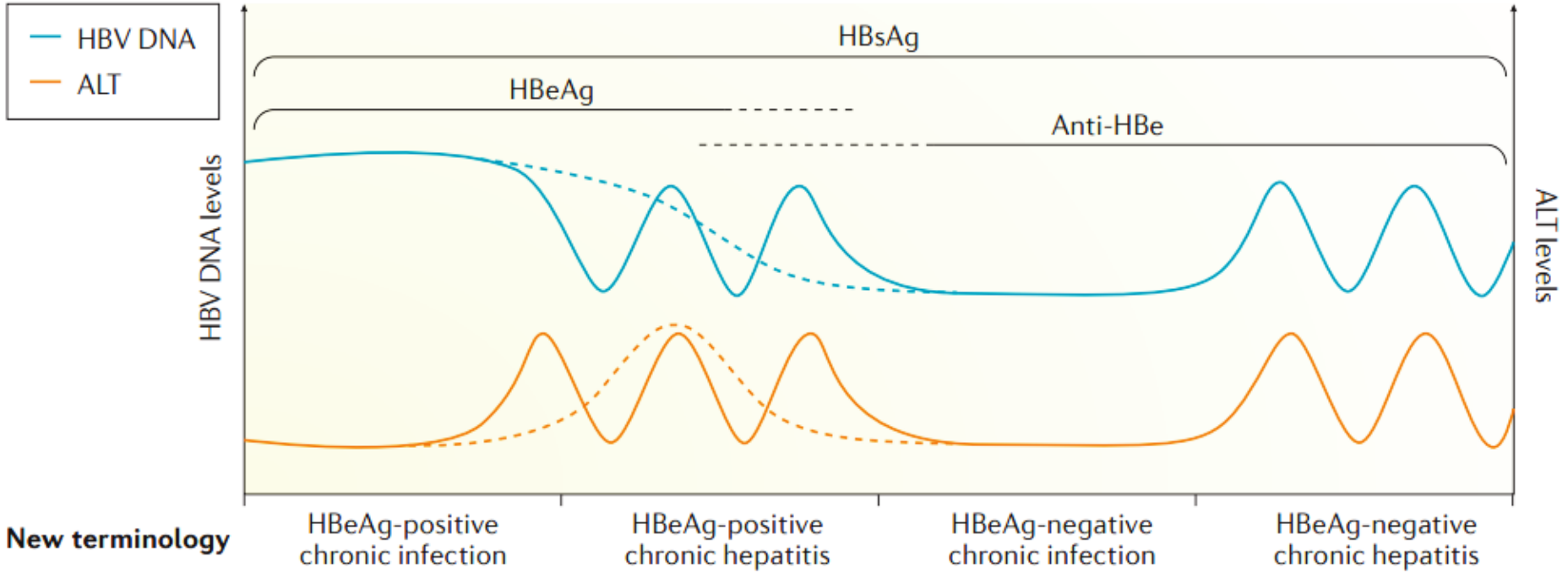
HBV chronic stages



- active replication high levels of voral DNA <u>but</u> HBeAg negative = **precore** variant
- ALT ↗, hepatic fibrosis
- low rates of spontaneous disease remission

Yuen et al. *Nat Rev Disease Primers* (2018)

HBV chronic stages



5th stage (occult HBV infection) : HBsAg-negative (+/- anti-HBs)

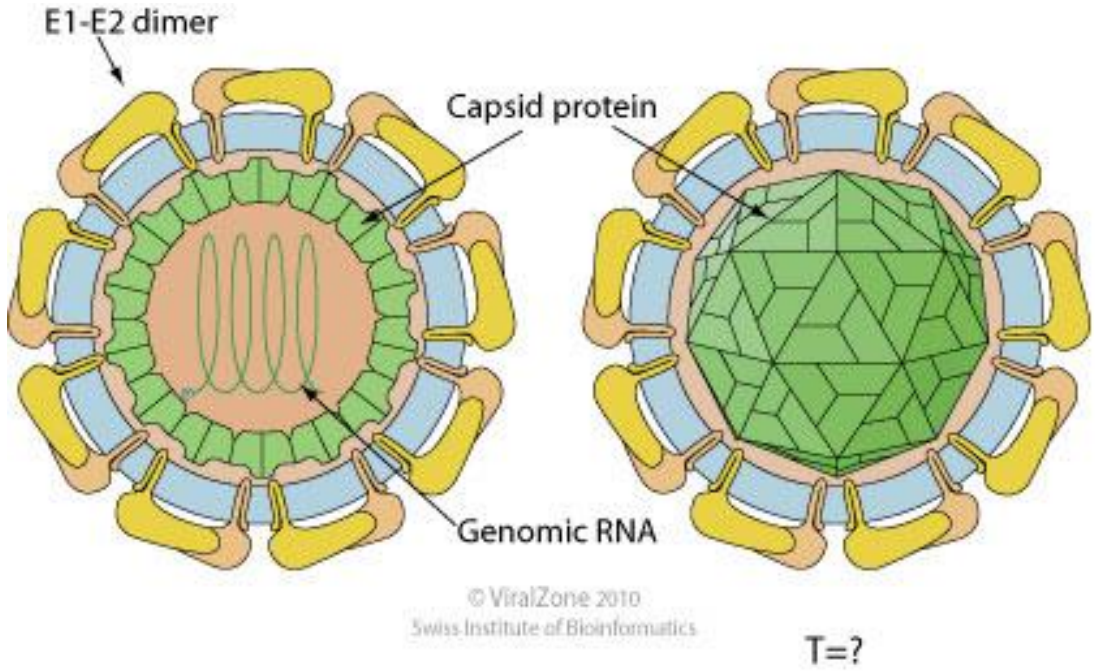
- ALT usually N
- undetectable HBV DNA (but HBV cccDNA in liver)
- possible reactivation if immunosupression

Yuen et al. *Nat Rev Disease Primers* (2018)

HEPATITIS C

Hepatitis C virus (HCV)

- Family: *Flaviviridae*
- Genus: *Hepacivirus*



- Structure: enveloped, spherical
- Genome: ssRNA (+), 9.6kb
- 8 genotypes,
- ≈90 sub-types, quasi-species
- hepatic tropism, infect humans and chimpanzees

History of HCV research

electron microscopy picture **2016**

Combination of DAA achieves 100% SVR rates **2013**

Identification of the complete set of receptors **2011**

2012 Licensing of Telaprevir and Boceprevir

Infectious HCV cell culture system **2005**

Functional HCV pseudoparticle system **2003**

Noninfectious replicon cell culture system **1999**

Infectious cDNA clone constructed **1997**

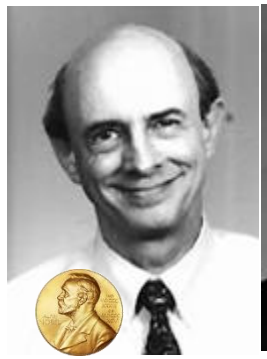
1998 IFN-alpha and ribavirin combination therapy

HCV serine protease structure (NS3-4A) **1996**

1993 Delineation of HCV genome organization and polyprotein processing

1989 **Identification of hepatitis C virus**

Description of non-A, non-B hepatitis **1975**


Alter

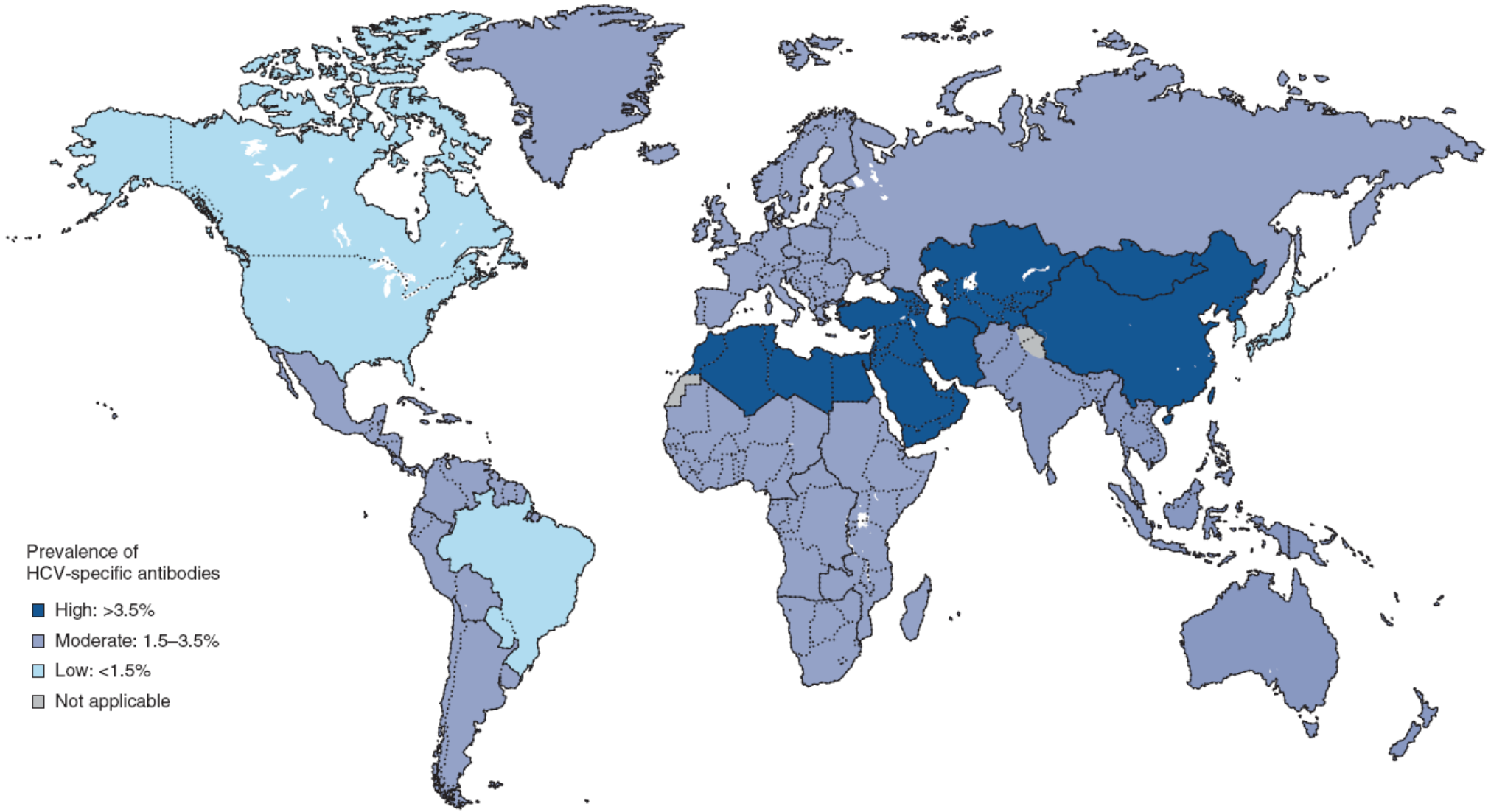

Houghton


Bartenschlager


Wakita

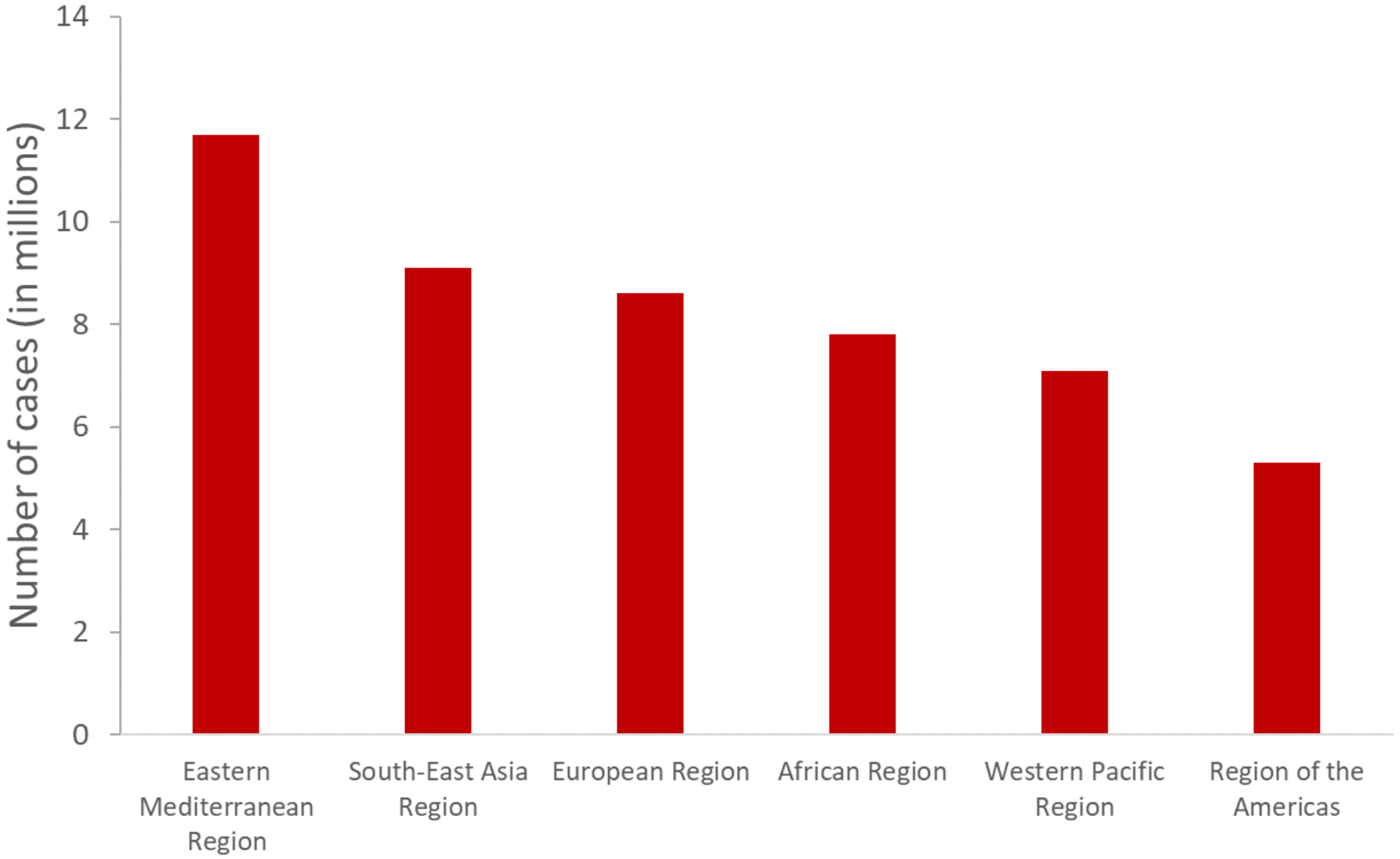

Rice

Hepatitis C: epidemiology



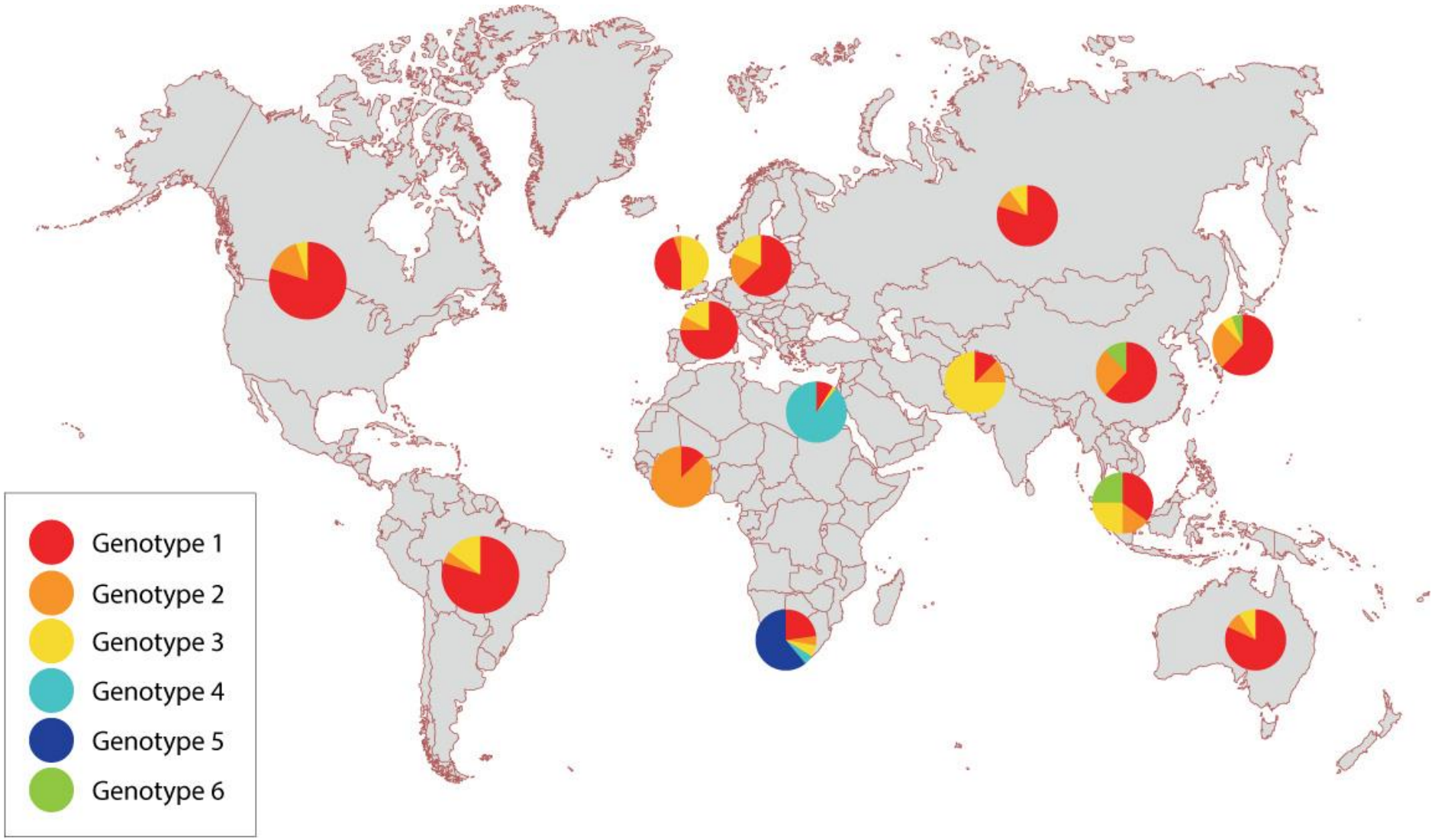
Thomas, D . *Nat Med* (2013)

Hepatitis C: epidemiology



GLOBAL HEPATITIS REPORT, 2024, WHO

Hepatitis C: epidemiology



Hepatitis C: epidemiology

- worldwide: **50 million** people with chronic hepatitis C
- estimation of 115 million people anti-HCV antibody positive
- **1 million new infections** in 2022
- **240 000 death** in 2022 (cirrhosis and hepatocellular carcinoma)
- 36% of people living with HCV are aware of their infection
- 20% treated with direct acting antivirals (12.5 million people)

Hepatitis C: epidemiology

- France (2011):
- prevalence: 0.75 % for anti-HCV antibodies (350 000 people)
- 200 000 people chronically infected by HCV

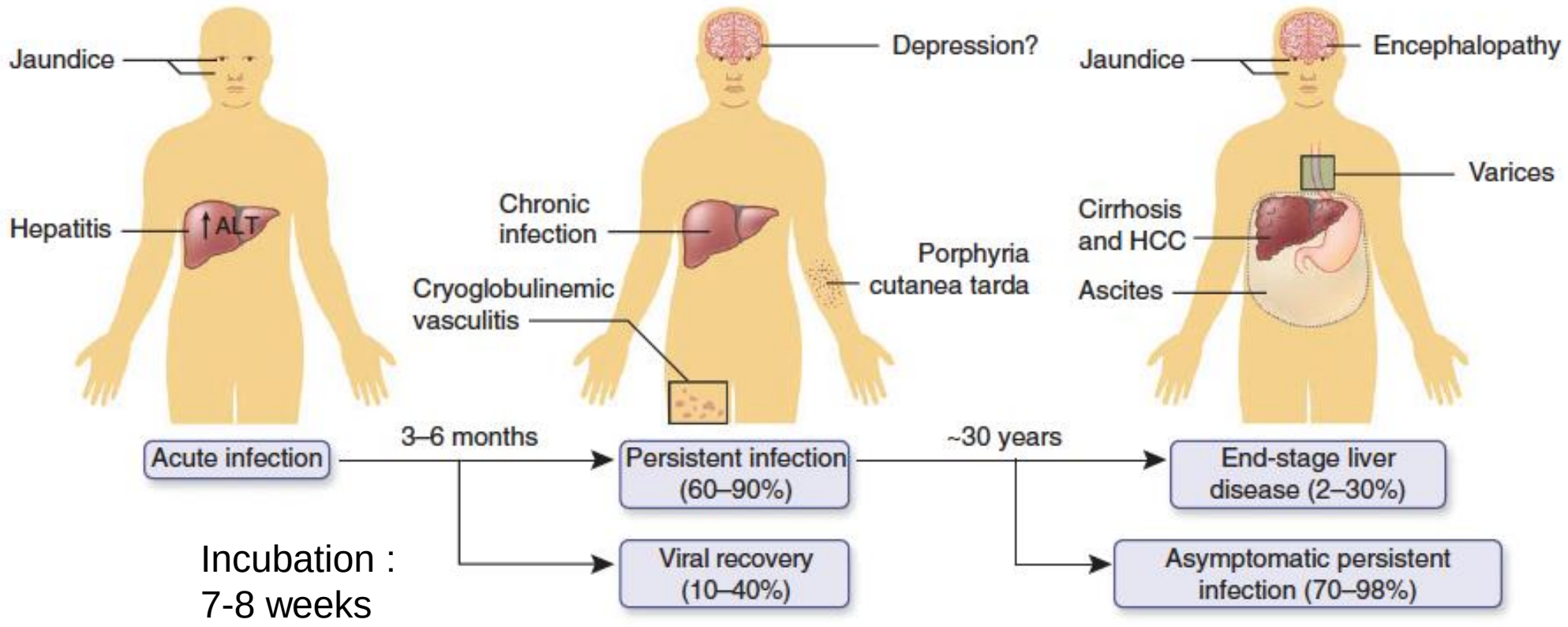
- “Barotest” study (2016) :
- prevalence of HCV RNA : 0.30%, meaning 133 466 people infected with HCV

HCV: transmission

bloodborne virus (requires blood-to-blood contact to be transmitted)

- injected drugs
- infected blood products or invasive procedures in health-care facilities with inadequate infection control practices (less important since 1990's)
- tattoo, piercing
- mother-to-child and sexual transmission (risk increased if HIV infection)

Hepatitis C: natural history of infection



Incubation :
7-8 weeks

chronic hepatitis +++ → Cirrhosis (15–30%)
Hepatocellular carcinoma: 2–4% per year in cirrhosis
one of the leading causes of liver transplant worldwide

+ Extrahepatic manifestations (Vasculitis, glomerulonephritis, lymphomas…)

Thomas, D . *Nat Med* (2013)

Hepatitis C: pathophysiology

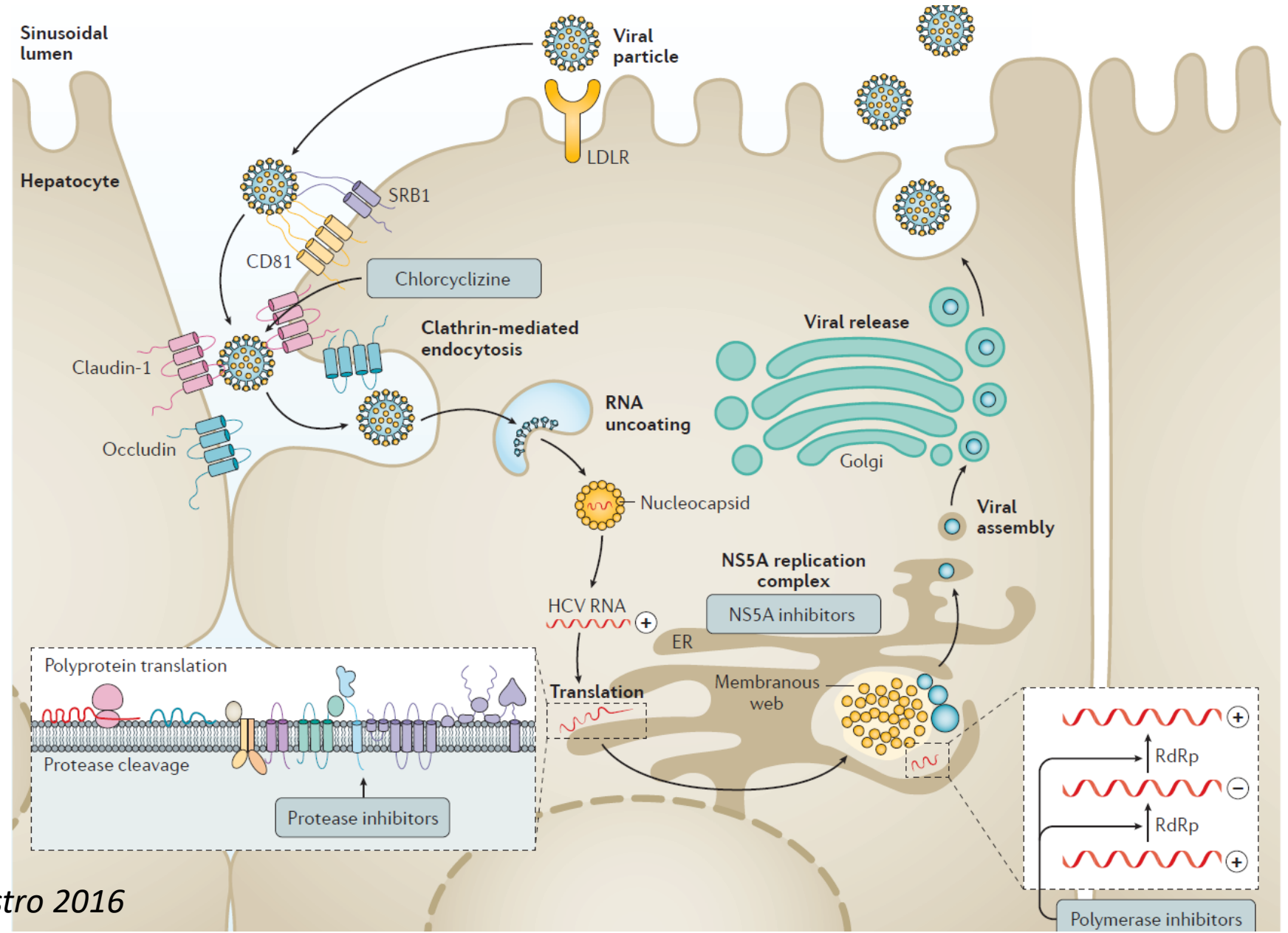
- Immune reaction against the virus causes liver damage
- HCV infection is associated with chronic inflammation and accumulation of lipids in the liver
- Seroconversion (anti-HCV antibodies) does not correlate with viral clearance
- **anti-HCV antibodies are not protective** (reinfection is possible)

- Factors that can impact the evolution of the disease:

➢ Positively: a proper immune response during acute infection (CD8+ lymphocyte in the liver)

➢ Negatively: alcohol, non-treated HIV infection

HCV: replication cycle

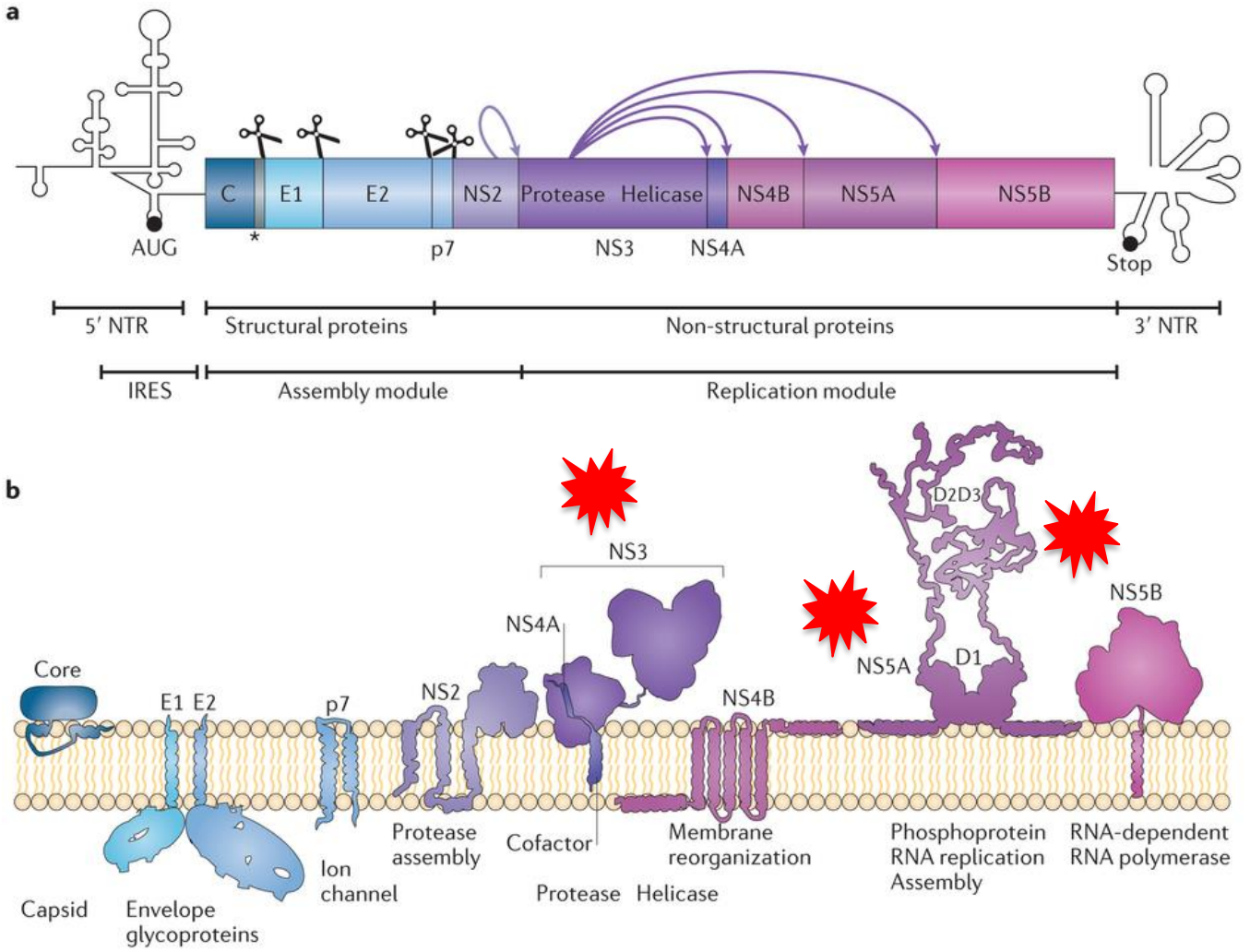


Götte et al nrgastro 2016

HCV: genome organization

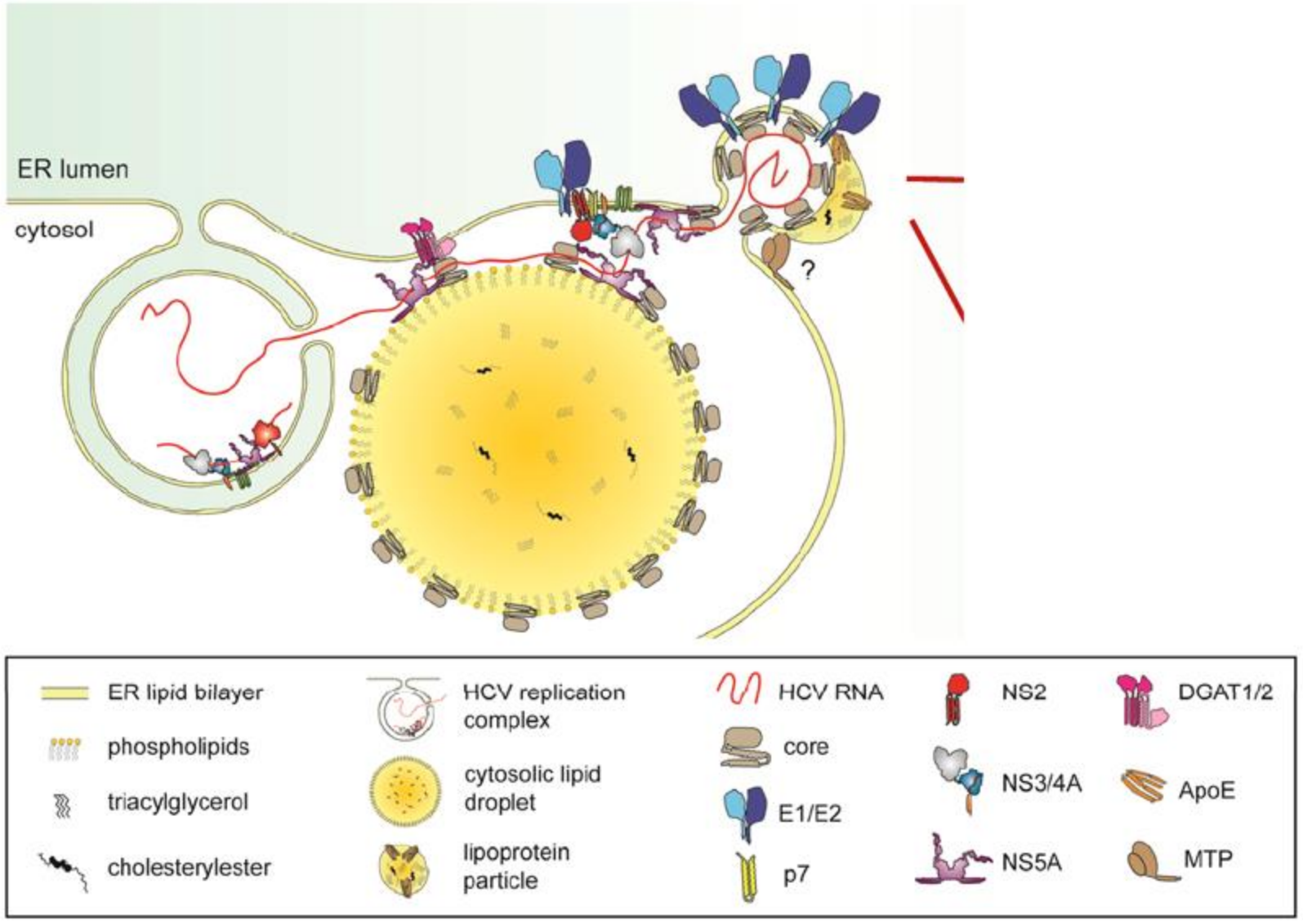
genome of 9.6kb translated in one polyprotein cleaved in 10 mature proteins by cellular and viral proteases

IRES: internal ribosome entry site



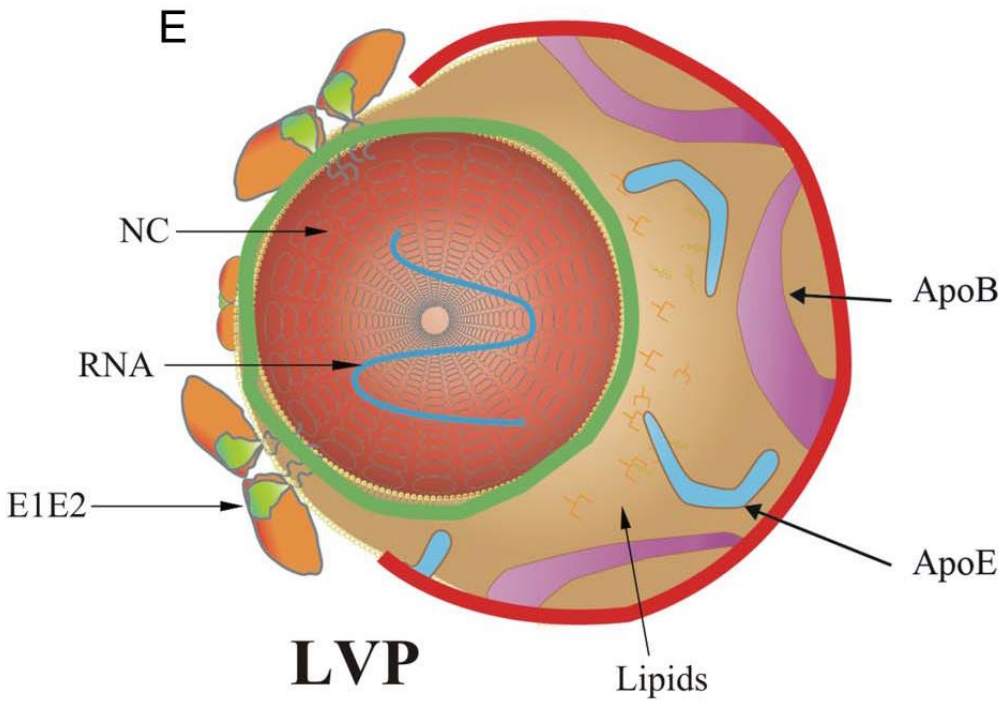
Nature Reviews | Microbiology

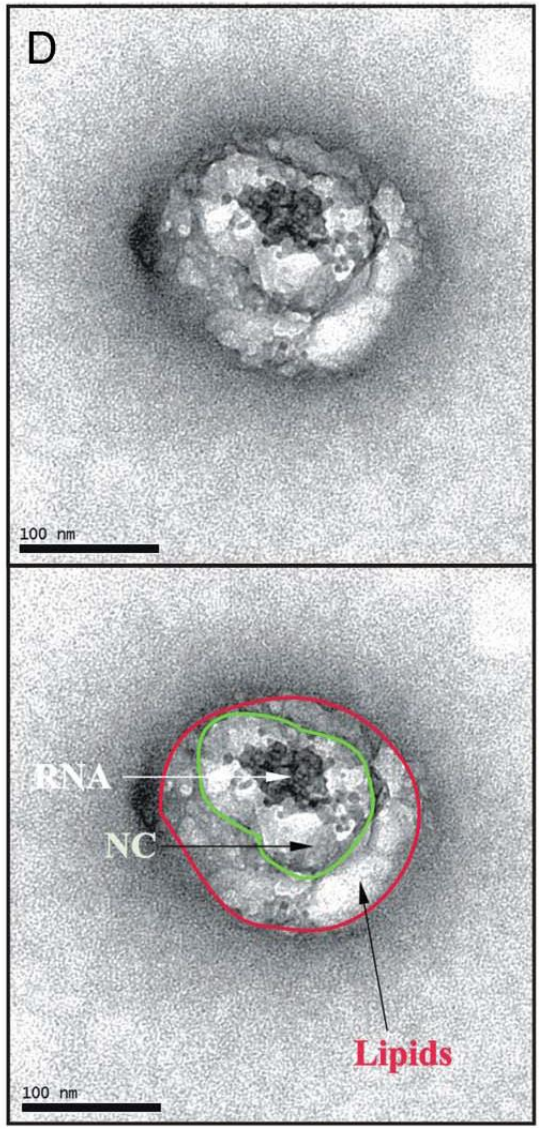
HCV RNA replication and assembly



Paul et al., CHM 2014; Lindenbach & Rice NRM 2013

HCV lipoviral particules





Hepatitis C: diagnosis

- Indirect: immunoassay

➢ anti-HCV antibody: RDT of lab-based assay (ELISA)

➢ if positive: contact with HCV

➢ Limits: late seroconversion (2-8 weeks), immunocompromized patients (seronegatives)

Hepatitis C: diagnosis

- Direct : HCV RNA nucleic acid test

(to confirm infection if anti-VHC +)

- ➢ Quantitative : RT-qPCR (important for monitoring treatment response)
- ➢ chronic infection is defined by HCV RNA persitence > 6 month

- or Core Ag detection

- genotyping? with the new treatments, genotyping is not required anymore in most cases

Hepatitis C : who to test?

- focused testing :
- Adults and adolescents from populations most affected by HCV infection (population with high HCV seroprevalence or history of exposure and/or high-risk behaviours for HCV infection)
- clinical suspicion of chronic viral hepatitis
- General population testing (intermediate and high seroprevalence)
- Birth cohort testing

- Blood donor (HCV RNA in France)

Hepatitis C: how to test?

anti-HCV antibody
RDT or laboratory-based immunoassay

(-) → no contact with the virus

(+)

current acute or chronic HCV infection OR past HCV infection

detection of HCV RNA (RT-PCR) or Core antigen

(-) → past HCV infection

(+)

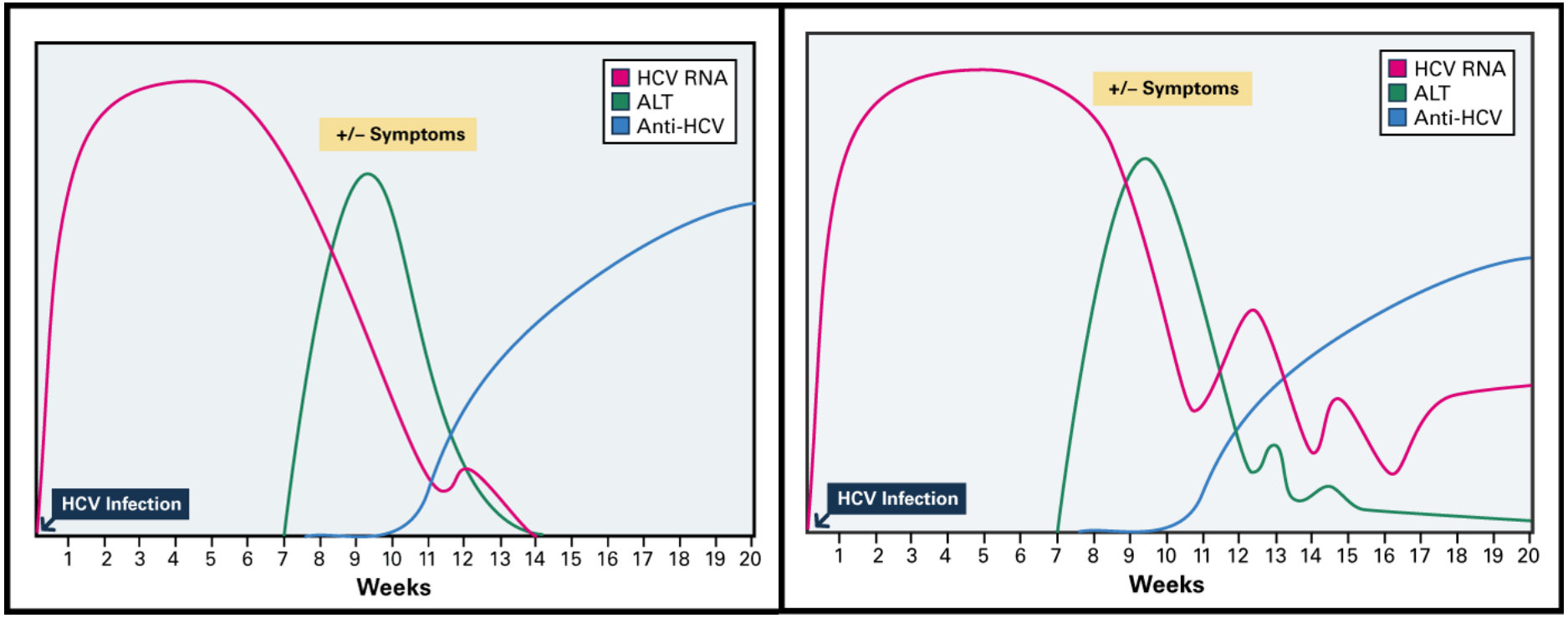
HCV infection (chronic if ARN + more than 6 months)

assessment of stage of liver disease, comorbidities, co-infections (HIV, HBV, HAV)

Hepatitis C: evolution of markers

resolved acute hepatitis C

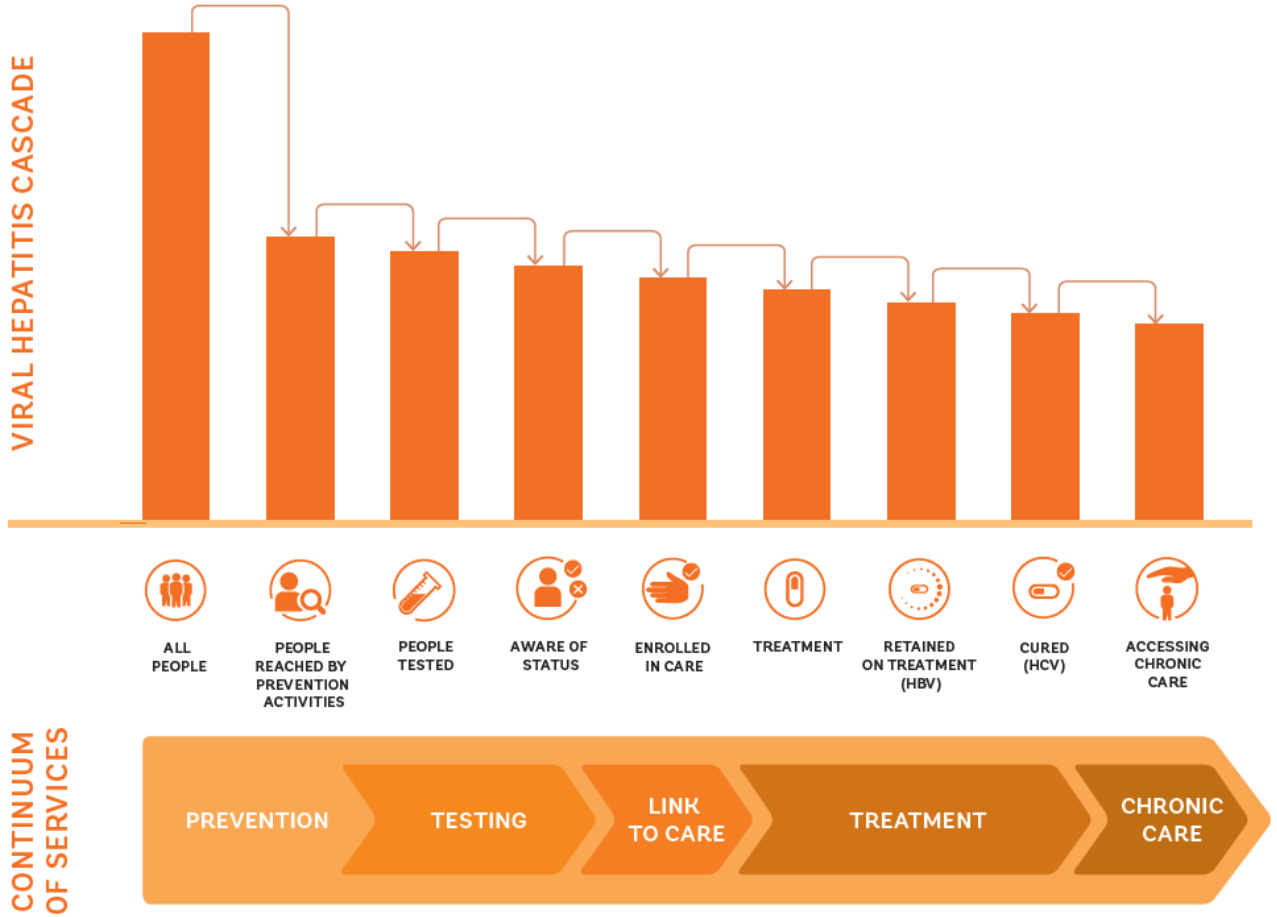
Chronic hepatitis C



hepatitis C: only viral chronic disease to date that can be completely cleared from the organism (no latency or reactivation)
BUT reinfection is possible….

Global strategy on viral hepatitis

elimination of viral hepatitis as a public health threat by 2030 : **reducing new infections by 90% and mortality by 65%**



Source: Global health sector strategy on viral hepatitis 2016–2021. Geneva, World Health Organization; 2016 *(16)*.

PREVENT – TEST – TREAT

Prevention:

- safer health care procedure (injection)
- screening of blood donors
- Harm reduction (distribution of syringe and needles for people who inject drugs)
- HBV : vaccine (recombinant HBsAg)
- STI prevention

Treatment:

- HCV : combination of pangenotypic direct acting antivirals. WHO target = 80% of diagnosed treated in 2030
- HBV : interferon therapy or RT inhibitors. WHO target = 80% of diagnosed who are eligible treated in 2030